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A CAUSE OF APPENDICITIS AND OTHER INTESTINAL LESIONS IN MAN AND OTHER VERTEBRATES.

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Observations on Birds.

OUR observations on a large number of recently dead or dying grouse impels us to believe that in many cases death is primarily caused by the presence of parasitic worms, either Cestodes or Nematodes in various parts of the alimentary canal.

CESTODA.

Conspicuous amongst the entozoa of the Grouse is *Davainea urogalli* (Modeer), which in the grouse is only found in the small intestine. This is the tapeworm known to sportsmen and to keepers; it indeed frequently protrudes from the hinder end of the alimentary canal and sometimes trails like a pennant behind a bird that is flying. Besides this we have very frequently a second genus and species of Cestode the *Hymenolepis microps* (Diesing) which we have for the first time recorded from the grouse, this occurs in the duodenum with a third species *Davainea cesticillus* (Molin), but as we have only found this twice it may be neglected in a consideration of the effects of the cestode parasites upon the health of the birds.

In this enquiry I propose to confine myself to the action of entozoa on the wall of the alimentary canal and having given a short, preliminary account of what happens in the grouse to consider the evidence which is accumulating of injury done to the human intestine, caecum and appendix by the presence of entozoa.

Davainea urogalli (Modeer).

Almost all grouse contain *D. urogalli,* only a very small percentage being free from it. Although the body is large the head is extremely small but capable of expanding and contracting. Its average diameter may be put at about 0·16 mm. This head is provided with a protrusible rostellum which bears a double crown of very minute hooks, about 6·7 μ in length. There are also four well-developed suckers each bearing a large number of hooks varying from 6·6 μ down to half that size. In both cases the hooks are bent and are very sharply pointed, thus the whole forms an admirable weapon for injuring the mucous layer of the alimentary tract.

We have not as yet found that in the grouse *D. urogalli* has set up any very marked lesions or that it has caused very serious disease, but that at times it is capable of doing so seems most probable from analogy with a closely allied species which causes the so-called "nodular disease" of the intestine so fatal to poultry. This is caused by a closely allied tapeworm *D. echinobothrida*[1] (Mégnin, 1880) which in many respects is very like *D. urogalli.* Piana (1880) has figured transverse sections of the intestinal wall of a fowl which was infested with this species[2] and he shows how the tapeworm heads burrow right through the mucous lining of the intestine, entirely breaking its continuity, and come to rest in large vesicles filled with an exudate in the muscular sheath of the wall just below the serosa.

Moore (1895) has given a full account of the disease associated with these nodules, which vary in size from being scarcely visible to having a diameter of 4 mm. Moore states that they are either circular or lenticular and over the larger ones the mucosa sloughs leaving small ulcerated depressions. These also contain an exudate, a greenish-yellow necrotic substance and surrounding this a thin layer of infiltrated tissue. The smaller nodules contain a more purulent substance. His sections showed, as did Piana's, that the heads of the tapeworms had penetrated the mucous membrane and were situated in different layers of the intestinal wall. Though more difficult to detect in the larger and therefore older nodules they were almost invariably

[1] Ransome (1905) regards this species as distinct from *D. tetragona*, a common parasite of fowls, partly on the ground that it causes disease and *D. tetragona* does not.

[2] He called them *Taenia botrioplitis* but it is the same species.

found in the smaller and younger swellings. In the earlier stages a cell-infiltration envelopes the head of the *Davainea* in the nodule. Moore dwells upon the wide prevalence of this disease and the chances of its being mistaken for tuberculous disease in fowls. He states that it "is highly probable that the total loss it occasions both from deaths and from the shrinkage of poultry products, due to the chronic course of the disease it produces, is very large."

Hymenolepis microps (Diesing).

We found the second tapeworm, which exists in any abundance in grouse, in September 1905. It had hitherto escaped the notice of the numerous observers who have for years been working at grouse disease. Its name is *Hymenolepis microps* (Diesing, 1850) and it lives in countless numbers in the duodenum, yet it is unrecognizable when alive. In this state the contents of the duodenum resembles a thick purée. If to this purée we add corrosive sublimate the tapeworms, which are so transparent when alive as to be invisible, slowly whiten and reveal themselves as countless fine opaque threads each with one end—the head end—sunk in the walls of the alimentary canal. *H. microps* is a very fine, fragile but long, worm attaining at times a length of 15 cms. and it consists of an enormous number of proglottides. The head like the head of *D. urogalli* has a rostellum and four suckers, but the rostellum is alone armed with hooks. These are very numerous, some 16 μ in length, very sharply pointed and shaped like slightly curved bayonets. In transverse sections of the duodenal wall of a grouse infested with these tapeworms, one sees the head "nuzzling" down between the villi.

Nematoda.

Passing to the thread—or round—worms which infest the alimentary tract of grouse, here again we find three species, one of which, *Syngamus trachealis* von Siebold, is so rare as to be negligible; another species *Trichosoma longicolle* Rudolphi is so difficult to see that all previous observers overlooked it, and we ourselves did not find it for months, although when once found we had little difficulty in finding it again and again; and a third species *Trichostrongylus pergracilis* (Cobbold) known to the older observers and the apparent cause of profound disease in the grouse.

Trichosoma longicolle Rudolphi.

T. longicolle lives in the duodenum and upper end of small intestine (jejunum), sometimes alone, sometimes associated with *H. microps.* They are not very evidently associated with grave disease, but when they are present they seem to cause a great destruction of the lining epithelium which even in birds just killed and quite warm is cast off in large clumps and masses. This form like its near ally *Trichocephalus trichiurus*[1] of the human intestine has an extraordinarily fine head and neck, hardly much greater in diameter than one of the epithelial cells lining the grouse's duodenum and this could easily, and we believe does at times, pierce the wall of the intestine and so let out bacteria, harmless enough in the alimentary tract but capable at times of exerting a pathogenic action when they reach the tissues of the intestinal wall or the peritoneal cavity.

Trichostrongylus pergracilis (Cobbold).

The second common Nematode of the digestive apparatus of the grouse is the *Trichostrongylus pergracilis* found in the caeca, and here it should be mentioned that the caeca form a large and very important part of the digestive apparatus in a grouse. Together they are at least as long as the whole of the rest of the alimentary tract, and in them absorption of the digested food takes place. When the caeca of the grouse contain a large number of the *T. pergracilis* its tissues undergo profound changes. The pathological changes associated with the presence of these worms are still the subjects of investigation but that the presence of the worms is intimately associated with grave disease, there can be no doubt.

As was the case with the *Trichosoma* of the duodenum so is it with the *Trichostrongylus* of the caeca. Both when alive are as transparent as a *Sagitta.* For a long time we could only detect them after the addition of some such reagent as corrosive sublimate when they became opaque and visible. Latterly Dr Wilson has devised a simpler method of verifying their presence. It is to press a thin film of the caecal contents between two microscopic slides and to hold this up to the light, then if any worms are present they stand out as transparent lines against the background of the semi-transparent chyme.

[1] = *Trichocephalus dispar* Rudolphi, 1801.

Syngamus trachealis von Siebold, 1836.

Another case of the continuity of the lining membrane of an organ being destroyed is that caused by the *Syngamus trachealis* found in the trachea of poultry and pheasants. This so-called red- or forked-worm pierces through the wall of the trachea and actually clenches the teeth with which its mouth is provided in the cartilaginous tracheal rings. So close is the attachment that the body of the worm will rupture if when once firmly fixed attempts are made to pull it away from the tracheal wall. If the trachea contains septic organisms and the cartilage were easily infected by them a more efficient inoculating medium could not be devised.

Observations on other Vertebrates.

Before passing on to consider the relation of the nematodes to the wall of the human intestine and caecum I should like to draw attention to one or two striking cases of lesions caused by thread-worms in other Vertebrates.

THE HORSE.

Such an instance is the *Sclerostoma equinum* so often found in the colon and caecum of the horse. This nematode pierces the mucosa until it reaches the capillary blood vessels and then engorges itself upon the horse's blood. The walls of the alimentary canal infested with this parasite are dotted with small reddish ulcers which heal sooner or later according to the nature of the bacteria which have access to them. When the bacteria are pathogenic the ulcers are formed at the place of the lesion and here various forms of microbes are found[1]. These ulcerations can according to Weinberg extend until they attain an area of 23 mm. × 8 mm. In them the mucosa and often the sub-mucosa is destroyed and a marked infiltration of leucocytes, amongst which many bacteria occur, takes place in the deeper layers of the sub-mucosa. In other cases the ulcers are replaced by small abscesses some of which attain a considerable size (85 mm. × 33 mm.); they contain a fluid but there is little infiltration of leucocytes. These ulcers are especially common in horses that are wasting away and are attributed by

[1] Faure and Marotel (1902). I have not been able to see this Paper but I am indebted for a summary of it to Weinberg's article (1907). From this I have taken many references and many statements.

Weinberg to an infection by some peculiarly toxic, anaerobic bacteria. The very severe mortality amongst horses due to the presence of *S. equinum* is thus accounted for. The question where do the bacteria come from has been advanced a stage further by the researches of Weinberg and Saeves. They have succeeded in withdrawing the contents of the intestine of 97 Sclerostomes taken from 25 horses. Thirty-three of these worms contained bacteria in the contents of their alimentary canal: *B. coli*, "*Enterococcus*," and a *Diplobacillus*, whilst cultures yielded *Streptococcus* and *Staphylococcus*. It is thus evident that the *Sclerostomes* can in many cases infect the pierced tissues not only by chance bacteria sticking to their exterior but also by the contents of their intestine should they escape.

Man.

With reference to bacteria adhering to the outside of entozoa and by them conveyed to places where they become pathogenic it may be mentioned that Piana was the first to show the migration of *Cysticercus pisiformis* into the liver of the rabbit. Metchnikoff (1901) first drew the attention of medical men to the danger which the presence of entozoa in the human intestine entailed upon their hosts. In the Harben Lectures (1906), he relates instances in which attacks of appendicitis have been associated with the presence of *Oxyuris* and *Trichocephalus* in the alimentary canal. Guiart and Grimbert (1906), further consider the matter in some detail. They consider that entozoa, especially round-worms, act as inoculating needles, and play a part in the etiology of certain diseases of the wall of the alimentary tract and of the liver comparable to that played by certain Diptera and Ixodoidea in the diseases of the blood. The gravity of the disease set up has of course a definite relation to the virulence of the bacteria admitted to the deeper tissues, and the course of the disease runs on quite independent of whether the inoculating needle—the entozoon—has been removed from the intestine or not, but removal naturally stops further infection.

If we now consider in turn the effects that three of the commonest human nematodes, *Oxyuris vermicularis*, *Ascaris lumbricoides* and *Trichocephalus trichiurus*, have upon the walls of the places they live in we shall find that the part played by these entozoa is being daily better appreciated.

Oxyuris vermicularis (Linnaeus, 1767).

O. Seiffert (1908) draws attention to the lesions in the mucous membrane of the rectum caused by these very common worms and to the fact of the intestinal catarrh they frequently set up. Wagener (1904) found amongst the Peyer's patches of a hog, five years old, 15—20 small nodules which when investigated microscopically revealed the calcified remains of *Oxyuris* worms. He considered that the worms had penetrated into the follicles, formed ulcers there, and when the ulcers healed had undergone calcareous degeneration. Ruffer (1901) also records a number of tumours in the rectum of a man, the tumours varying in size from a pin's head to a walnut. The tumours contained ova of *Oxyuris*; since these could not have got there by themselves the probability is that they were laid *in situ* by some female which had penetrated the rectal wall. Fröhlich is quoted by Weinberg as describing a case in which he found 16 *Oxyuris*, all females, living surrounded by pus in a tumour in the peritoneum of a child of 11 years of age. Edens (1896) found the head of an *Oxyuris* in a nodule of a Peyer's patch in a child of seven years whose intestine presented the typical lesions of primary, intestinal tuberculosis. There are many more examples, but these seem to me sufficient to show that *Oxyuris* can and, not unfrequently, does perforate the wall of the alimentary canal.

Relation of entozoa to Appendicitis in Man.

The relations of this worm with appendicitis may now be considered. The worms live in the lower part of the small intestine, in the caecum and in the appendix vermiformis. When the eggs begin to develop in the fertilized female the worms leave the caecum and appendix and passing through the colon arrive at the rectum; here they may lay their eggs but most of them creep out of the body to lay them elsewhere.

Galli-Valerio (1903) has described a case of an appendix which had been perforated and which contained many Oxyurids, the tail of one of the male specimens was threaded through the mucosa and microscopic sections showed spaces resembling the perforation which were surrounded by an infiltrated zone infected with bacteria. Weinberg (1906, 1907) gives at length an account for which he is indebted to Dr Thevenard of a boy aged 11 years, who was after much suffering operated on for appendicitis. On examining the appendix about 1·5 cm. from the free end

a small nematode—which proved to be a female *Oxyuris*—was found threaded through the mucosa and so firmly fixed that light traction at either end failed to dislodge it. The appendix was congested and the congestion was most pronounced in the neighbourhood of the parasite. Microscopic study of sections showed that the worm had pierced through the mucosa, traversed a gland, and reached the vascular layer in the submucosa. These sections also showed a very inflamed condition of these parts, the parasite was surrounded by polynuclear leucocytes and amongst them a large number of bacilli. There were also signs of lymphangitis around the same spot extending into the sub-serous layer and here the blood vessels were very congested. All these disturbances had their centre in the impacted *Oxyuris*.

Seiffert (1908) in his summary of the relation of entozoa to appendicitis quotes the following:—Still (1899) recognizes Oxyurids as the great cause of catarrhal affections in the appendix: Moty (1902) recognized Oxyurids as the cause of three of his cases of appendicitis: in Morkowitin's (1902) cases numerous Oxyurids were obviously the disposing factors to appendicitis: Ramstedt (1902) found a regular tangle of *Oxyuris* in an extirpated appendix and believed that they had set up the inflammation: Oppe (1903) found *Oxyuris* in six appendices and thought that a "Wurmkur" should be considered in cases of appendicitis—this is especially indicated when examination of the faeces reveals the ova of *Oxyuris* or *Ascaris*; *Trichocephalus* is much less affected by antihelminthics:—Hanau (1903) communicates a case where without doubt *Oxyuris* set up appendicitis: von Bégonin (1902) a case in which in an extirpated appendix he found the mucosa ulcerated and 15 *Oxyuris* in the lumen and Putnam (cited by Spieler (1904)) one in which 20 specimens were found: finally Schöppler (1906) holds that the danger of appendicitis is not removed when an *Oxyuris* which has wandered into the appendix dies; this is obvious if before death the worm has pierced the linings of the part and given exit to bacteria which may then exert a pathogenic action.

Ascaris lumbricoides (Linnaeus, 1758).

This, one of the commonest—as it is one of the largest—of the nematode human entozoa, normally inhabits the small intestine, but it is a little apt to wander and has in fact been found all over the body. It occurs at all ages, but is as a rule commoner in children about half

grown, and it is found in all climates, though it is more abundant in warm climates than elsewhere.

The genus *Ascaris* has, in certain of its species, the power of attaching itself to the inner lining of the wall of the alimentary tract. Guiart has described specimens of *A. conocephalus* from the intestine of a dolphin: "profondement incrusté dans la muqueuse, s'y était taillé une sorte de cupule assez profonde," and Weinberg has reported the case of an Ascarid lightly attached to the duodenal mucous layer of an ape and at this point there was ulceration. The latter writer quotes a letter from Dr Fontoynot, Professor at the School of Medicine, Tananarivo (Madagascar), in which he says that *A. lumbricoides* is of extreme frequency in the natives (Malgache), in whom, among other troubles, the worms frequently set up a mild appendicitis. He states "chaque fois que j'ai vu un indigène présenter du météorisme abdominal, de la péritonie légère, ou mieux du péritonisme avec localisation manifeste de la douleur au point de MacBurney, et epâtement dans la fosse iliaque droite, la santonine prise à la dose de 0·15 gr. a toujours fait évacuer un plus ou moins grand nombre d'ascarides et, par ce fait, a toujours amené la cessation de tous les phénomènes appendiculaires." The further fact that he states that with one exception he has not met with grave appendicitis amongst the natives, to some extent explains the immunity of the highly parasitized Chinese, an immunity which has led Martignon (1901) to doubt whether entozoa play any part in setting up appendicitis. Weinberg also recounts an observation made by Aldo Castellani on the extirpated appendix of a young girl, into which an *Ascaris* had penetrated and in which one half of its body was firmly fixed. Between the parasite and the walls of the appendix was a purulent fluid charged with *Bacillus coli*. Other cases of the impaction of the worm are recorded by Kelly and Hurdon (1905): by Bergmann (1890), in which case the *Ascaris* had bored through the walls of the appendix and attained the perivisceral cavity: Arboré-Rally (1900) regarded a severe case of appendicitis in a boy of 10 years as due to Ascarids: Triboulet (1901) regarded another case as due to Ascariasis: Schiller (1902) states that the disappearance of certain caecal abscesses after the expulsion of Ascarids supports the view that they were the cause of the disturbance and in this tends to confirm the views previously expressed by Czerny and Heddäus (1898); Schwankhaus (1901) found in the peritoneal cavity of a boy of 13, who had died of diffuse peritonitis, an *A. lumbricoides*, which had bored its way through from the appendix: Nason (1904) described a case in which an *Ascaris* inside the appendix had so coiled

itself and the appendix (like a finger in a glove) around the intestine as to cause an obstruction: Page (1906) records an operation on a man in whom appendicitis had been diagnosed, which revealed a number of Ascarids in the body cavity, and the specimens of this worm continued to make their way through the wound even eight days after the operation: other cases might be quoted, but I think enough has been said to show that in some cases *Ascaris lumbricoides* is an etiological cause of appendicitis and peritonitis.

Trichocephalus trichiurus (Linnaeus, 1771[1]).

Of all the common parasites in the human alimentary canal this is the one most generally recognized as causing appendicitis. Its normal habitat is the caecum and the colon, but it is found, though more rarely, in the vermiform appendix and in the small intestine. It occurs, with the exception of sucklings, in persons of all ages. It is cosmopolitan in its distribution, but is less common in the colder regions, though common in temperate climes. Braun (1908) states that dissection shows it to be present in the body in the following per cent. of those investigated in various places: Kiel 31·8 %, Munich 9·3 %, Göttingen 46·1 %, Basle 23·7 %, Greenwich 68 %, Dublin 89 %, Paris about 50 %, and in Southern Italy almost 100 % of the people are infected. Its presence as determined by the presence of the eggs in the faeces gives—where they are comparable—slightly different figures: Kiel 45·2 %, Munich 8·26 %, London 7·8 % and Switzerland over 50 %[2].

Although the worm has been known, at least since the time of Linnaeus, but little has been done until recently to investigate its relations with the wall of the alimentary canal in which it lives. Askanazy (1896, p. 104) found that the *T. trichiurus* fed upon blood and that the only means of getting its food must be by piercing the mucosa. Wichmann (1889) made a painstaking examination of the subject, and his conclusions were that although the worm was so

[1] = *T. dispar* Rudolphi, 1801.

[2] These statistics date from some years ago and are probably not accurate for the present date. More recently French and Boycott (1905) found 7·8 % of infection in 500 in-patients of Guy's Hospital, varying in age from a babe to over seventy. 84 % of infections fell between the ages of five and forty, and of the patients examined between these years 11·75 % were infected. I feel bound to add that in the opinion of these observers their research affords no support "to the notion that *Trichocephalus* has any aetiological relationship to appendicitis."

attached to the inner face of the intestinal wall that it required some slight force to withdraw it, this was due to its head-end being sunk in the mucus and coiled or wrapped round the villi. He found no evidence of lesions nor any solution of the continuity of the mucosa. Since Wichmann's time methods of research have improved and attention has been more closely focussed on the problem. Weinberg, whilst allowing that in many cases the whip-like fore-end of the body is simply hidden in the mucus, maintains also that "il y a des trichocéphales qui sont si bien fixés qu'en essayant de les détacher ou arrive plutôt à séparer le tronçon terminal de leur partie antérieure." In fact he maintains that the whip-worm is always fixed on the mucosa, and certainly some specimens we have at Cambridge confirm this statement. At times the anterior end passes through the mucosa and appearing again as a needle may be threaded through a curtain, at other times it hid its anterior end in a canal burrowed out in the mucous lining. Girard (1901, p. 265) has recorded finding two whip-worms in the extirpated appendix of a girl of eight, one worm had penetrated the mucosa and there was much inflammation about the lesion, numbers of mono- and polynuclear leucocytes and a copious bacterial flora were aggregated there. A similar inflammatory centre, surrounding the point of entrance of a whip-worm into the mucous layer of an idiot dead at the Vaucluse Asylum, has been described by Vigouroux and Collet (1905, p. 270). Kaposi (1902) attributes a case of an appendicitis to the intervention of *T. trichiurus*. Moore (1906, p. 364) has recorded a case of appendicitis in which a "small worm was found"..."identified by Dr Thursfield as *Trichocephalus dispar*." Oui (1906) found two specimens with their heads deeply embedded in the mucous layer in another appendix, and Kahane (1907) communicates the case of an appendix which on examination showed inflammation and in which were a number of specimens of *T. trichiurus*, some free and some embedded in the mucosa.

Amongst the most interesting cases are some recorded by Weinberg on the presence of nematode parasites in apes and monkeys. These animals are very subject to parasites and are very frequently infested with *T. trichiurus* as are also Lemurs. He gives a figure of the interior of the caecum of a *Macacus cynocephalus* which is riddled with scores of specimens of this worm. The monkey died after two days of fever and was at once examined, when all the organs were found congested. The caecum and colon contained hundreds of whip-worms, and histological investigation showed that the points of fixation of these worms were centres of inflammation which extended deeply into the wall of the

intestine and contained many polynuclear leucocytes and coli bacilli. The same microbes were obtained in cultures made from the blood. Weinberg considers the monkey died of an infection produced by a pathogenic organism introduced by the whip-worm into the walls of the intestine. He cites a further instance of a *Macacus sinicus* which died of septicaemia caused by *B. coli* and in whose intestine but one specimen of *T. trichiurus* was found, and he considers this one worm sufficed to bring about the fatal inoculation. The grave cases of anaemia described by many observers in cases of Trichocephaliasis may be due to similar inflammatory centres which as a rule are not visible to the naked eye but which are readily revealed on microscopic investigation.

Guiart and Grimbert (1906, p. 562) maintain that what they consider true of appendicitis (i.e. that the inflammation is set up by bacteria from the contents of the alimentary canal admitted to the tissues through punctures and perforations made by intestinal parasites) may also be true of Typhoid. They point out that if the bacillus of typhoid fever causes the disease entirely through its own efforts it is very difficult to understand why so small a percentage of people all drinking from the same water supply and all exposed to the same danger of wind or fly-borne infection suffer from the fever. If however the typhoid germs require a certain introduction to the walls of the alimentary canal one can understand its sporadic incidence and its association, formerly noticed, with the presence of *Ascaris* and *Trichocephalus* in the lumen of the intestine. An outbreak of typhoid occurred at Brest in the autumn of 1904. Investigating the dejecta of twelve patients suffering from the fever in the Hospital there Guiart found a constant passage of *Trichocephalus* eggs in ten of them. The number of eggs found in each case showed a strong infection. Of the two patients in whom there was not evidence that they were infected, one died and at the autopsy six specimens of *Trichocephalus* were found in his caecum. They may have been all males, or if females may have interrupted their oviposition; either alternative would explain the absence of eggs in the faeces. There was no opportunity of examining the caecum of the twelfth patient who apparently happily recovered, but his case may have resembled that of the man who died in whom the worms were found but whose dejecta showed no eggs. These numbers are too small to be conclusive and there seem to have been no control experiments, still they are at least suggestive. Guiart further states that renewed observation at Paris confirms the statement that *Trichocephalus* is

abundant in the intestines of typhoid patients except in children and that in them *Ascaris* seems to take its place as an inoculating agent.

The recognition of this association is no new thing. Roederer and Wagler in 1792 gave the earliest account of the " morbus mucosus " or typhoid fever, and they attributed the epidemic to the presence of the large number of intestinal worms (*Trichocephalus*) which on making autopsies were found in the alimentary tract. Pincl (1807) indicates that one should always suspect the presence of ' vers intestinaux ' in cases of fevers of the mucous lining. Davaine has further noted the association of typhoid and worms, and other observers to the same effect are quoted by Guiart and Grimbert (1906).

An interesting confirmation of Guiart and Grimbert's views as to the part played by entozoa in typhoid fever is found in the following experiment of Weinberg (1906). Typhoid bacilli were given to two apes, one of which quickly died of septicaemia, the other survived repeated doses of the bacilli for 33 days, during which time its temperature rose at evening from 38·9° C. to 39·6° C. but there was nothing characteristic in the temperature chart. When the ape died (33rd day) the post mortem showed in the ileum a number of ulcerated Peyer's patches which presented the characteristic features of typical typhoid lesions in various stages of their evolution. The lower end of the duodenum and the upper end of the jejunum of this ape were full of a mass of tapeworms, some of which were found fixed at the level of the ulcerations. The caecum and the colon contained a great number of *Trichocephalus*. Examination of the blood and of the spleen by cultures demonstrated the presence of the typhoid bacillus and microscopic investigation of the ulcerations in the intestine confirmed the presence of the same germ in its walls ; they also occurred in the small ulcerations which surrounded the point where the heads of the tapeworms were embedded. The authorities at the Pasteur Institute were satisfied that this was a true case of typhoid, and this is the more interesting as Grünbaum (1904) although he succeeded with an ape, failed to give a *Macacus* typhoid, though apparently Chantemesse and Ramond (1897) had succeeded previously. Soloukha morc recently working under the same conditions, and with *B. typhosus,* failed to convey the disease to an ape. Weinberg concludes that success in his case was due to the fact that the burrowing into the mucosa of the taenia's head and suckers afforded a port of entry for the germs to the tissues, where they set up the ulcerations, and he states that there were masses of the

bacilli at the points where the suckers of the tapeworms were attached to the intestinal wall. Weinberg concludes this part of his thesis by saying that his microscopic sections show that:

(i) The tapeworm by fixing itself on the intestinal mucosa, sets up an intense congestion at the point of fixation.

(ii) At the same time it applies to this point of the intestinal mucosa such bacteria as are to be found on its suckers, and on the other hand it imprisons, between its suckers and the intestinal wall, such bacteria as existed before on this portion of the mucosa.

(iii) A considerable number of leucocytes make their way to the surface of the mucosa and take up the bacteria.

(iv) At other times, the bacteria penetrate into the thickness of the mucosa and set up inflammatory changes which may end in one of those ulcerations which are often found at the point of fixation of the tapeworm.

It seems then that Weinberg does not allow that the Cestode head breaks the continuity of the mucosa. He does not give precise details as to the species of "ténia" he is dealing with and it may very well be that the unarmed species do not penetrate the lining of the intestinal wall. But whoever will study Piana's Paper will I think have little doubt that in such genera of tapeworm as *Davainea*, and I think we may add *Hymenolepis*, there is a solution of the continuity of the lining mucosa of the host.

We must also not leave out of account the fact that some people and races are much more "tolerant" of all sorts of parasites, bacterial and others, and when infected suffer far less than do others who are susceptible to their action.

I am not quite sure how much injury to the mucosa is required to admit germs which are harmless within the gut lumen, but pathogenic when they gain free access to the blood or tissues, especially when the latter have been injured.

Without doubt the passage of the bacteria which set up intestinal disease is immensely aided by any agent which causes a lesion in the mucosa. Such lesions are normally caused in man—apart from any irritating substances he may swallow with his food, such for instance as the powdered diamond or glass which is said to have been used in Italy in the palmy days of poisoning—by entozoa.

I have in this paper confined my attention in the main to but three human intestinal parasites, all of them nematodes. There are, however,

many more which merit discussion, but these three are, from my point of view, the most important. Two of these, the *Oxyuris* and the *Trichocephalus*, are comparatively common, and the latter is probably much more common than is usually recognized. I have given some figures above as to its prevalence. The family Doctor knows how common *Oxyuris* is. Comparatively few children escape it and it attacks the rich and the poor, the apparently well cared for and the neglected, with complete indifference. Only a couple of months ago I found three specimens of *Oxyuris* in the extirpated appendix of a patient who was quite ignorant as were her parents that she harboured these worms. Further I have confined my attention largely to appendicitis, there are, however, many other diseases whose presence is associated with entozoa in the alimentary canal, e.g. certain forms of diarrhoea; some of these have been described by Weinberg who has investigated the relations of many more parasites to the intestinal wall than are considered here. All tell the same tale.

With the discovery of bacteria and the important work which has been done during the last forty or fifty years the grosser human parasites have been rather left in the shade. Before that time it was much more usual to administer vermifuges from time to time. Many of the numerous ailments of children were treated by our medical grandfathers with antihelminthics, and even to-day Sir Patrick Manson recommends that in the tropics and in other places where the intestinal parasites are common a course of santonin should be administered to children every six months. In spite of the great increase in our knowledge and practice of Hygiene, care in our meat supply, etc. which has so materially lessened the number of cases suffering for instance from the pork- or beef-tapeworm, I am not sure that, as regards other entozoa, whose entrance into the body is less easily controlled, we keep the inside of our digestive system as clean as our ancestors kept theirs. But times are changing, and increasing attention is being paid to what I am convinced is a serious factor in certain diseases. The matter is one which in England has received so far but little attention. Looking through the list of my "cloud of witnesses" hardly an Anglo-Saxon name occurs. Our knowledge of the relations of the parasite to the intestinal wall is derived mostly from Italian, French and German sources. In the United States however there is at least one voice crying in the wilderness. In Professor H. B. Ward's (1907) careful consideration of entozoa as germ carriers and germ inoculators, he says

"there has prevailed during recent years among the medical men of this country an exaggerated idea of the unimportance of human parasites. This must now give way to a proper conception of the pathological significance of these organisms, based upon careful investigations of their actual influence upon the host."

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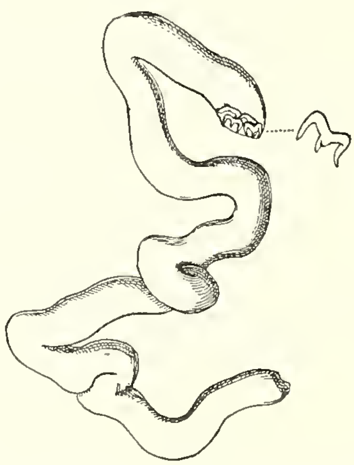
NOTE ON THE OCCURRENCE OF *TRIAENOPHORUS NODULOSUS* RUD. IN THE NORFOLK BROADS.

By A. E. SHIPLEY.

(1 Figure.)

A short time ago Professor Minchin of the Lister Institute of Preventive Medicine sent me the liver of a Perch, *Perca fluviatilis* Rond. which was infested with cestode cysts. On examination each of these proved to contain a single specimen of the larval form of *Triaenophorus nodulosus* Rudolphi. The perch was caught in Sutton Broad, Norfolk, and, as I know of no record of this tapeworm being found in this locality, I have prepared this short note on its occurrence.

The cysts were spherical or oval, the diameter being about 1—2 mm. The cyst-wall was thick and laminated. Within each cyst a larval form of *T. nodulosus* (see Figure) was coiled or tangled up so as to accommodate its length (some 8—10 mm.) to its narrow home. Anteriorly the characteristic hooks and the two suckers are visible. There was no trace of segmentation in the body.



Triaenophorus nodulosus Rud. larva × about 12. To the right one of the three-pronged hooks more highly magnified.

Zschokke[1] tells us that together with *Dibothriocephalus infundibuliformis* and the nematode *Cucullanus elegans*, *T. nodulosus* is one of the commonest of helminthes found in the fish of the Lake of Geneva. Like the first named of the above three worms it occurs in many species and in many individuals of those species. It further occurs all the year round. The cysts contain larvae in various stages of development. The cysts usually occur in the liver but they have also been found in the spleen, the muscles of the tail and in the walls of the intestine and in the body cavity, these last two situations harbour larvae in very early stages of development. They occur in some numbers, as many as thirty-six having been recorded from the same fish.

These larval forms attain at times a surprising length. Ordinarily from one to three centimetres long they have been found encysted in the tail muscles, eight, fifteen and even twenty-five centimetres in length.

These cysts have also been found in the tissues of the pike (*Esox lucius* L.) the normal host of the adult *T. nodulosus*, in the grayling (*Thymallus vulgaris* Nilss.), in the trout (*Trutta variabilis*) and in the salmon (*S. nubla*), but in the grayling, the trout and the salmon they attain very small proportions. Cysts have also been described from the pope fish (*Acerina cernua* L.).

The adult form normally inhabits the duodenum of the pike and lies with its head firmly fixed in the walls of the alimentary canal. It has also been found, though much more rarely, in the intestines of the fishes mentioned above.

[1] *Arch. Biol.* 1884, v. p. 153.

NOTE ON LEECHES SENT BY DR E. W. G. MASTERMAN FROM PALESTINE.

By W. A. HARDING, B.A.

These specimens were preserved in alcohol and contained in three bottles *a*, *b* and *c*.

(*a*) contained 1 large and 47 small leeches "out of infested springs at Safed, Palestine."

(*b*) contained a leech "extracted from pharynx of an Arab of Jerusalem."

(*c*) contained a leech which had been removed, by means of laryngeal forceps, from the vocal cords of a young peasant in Jerusalem.

All these, as their history led one to expect, proved to be examples of *Limnatis nilotica* Savigny, 1820.

This leech has often been inaccurately described and confused with other species and in determining the specimens before me I have followed Professor Blanchard, who alone has given a satisfactory diagnosis.

The following description applies to Dr Masterman's leeches and agrees with Blanchard's account of *Limnatis nilotica*:

Number and arrangement of eyes and of intestinal caeca, and position of genital openings, as in *Hirudo*. Posterior sucker of large size. Upper lip of anterior sucker divided on its inferior surface into two lobes by a deep longitudinal groove. Jaws covered by papillae and provided with numerous sharp teeth. [N.B. Blanchard makes more than 100 teeth: I make less than 100.] Inhabits stagnant water, particularly drinking places, and invades the throat and nasal fossae of man and beast.

Blanchard gives the colour and size of *Limnatis nilotica* as follows:

Dorsal surface reddish-yellow or greenish, generally traversed by four black lines and occasionally by a median yellow or green strip. Two lateral orange stripes. Length 100—150 mm., breadth 10—15 mm.

The leeches from Palestine, being preserved in alcohol, cannot be expected to have retained their original colours. Traces of the lateral orange stripes appear in some of the small specimens in bottle (*a*).

The large specimen contained in (*a*) could extend itself during life to at least twice its present length, that is, to a length of more than 100 mm.; the two examples taken from the human throat are probably about half grown.

Limnatis nilotica has a wide distribution extending from the Azores, through Northern Africa and Egypt to part of Western Asia. It is a species to which the term "Horse-leech" has been applied and it has been often confused with the European Horse-leech, *Haemopis* Savigny [= *Aulastoma* Moquin Tandon].

Savigny, whose figures are incorrect in certain respects, gives excellent drawings of this leech in a contracted and extended state and of the characteristic triangularly grooved lip referred to in my description of a leech from Angola [*Parasitology,* vol. I. p. 186].

A considerable literature exists relating to accidents caused by this leech, of a similar nature to those recorded by Dr Masterman.

Blanchard has collected much curious information on this point and gives many references.

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COMMUNICATION RECEIVED FROM THE SOCIETY FOR THE DESTRUCTION OF VERMIN.

95, Wigmore Street,
London, W.
July 7th 1908.

TO THE EDITOR
THE JOURNAL OF HYGIENE,
FETTER LANE, E.C.

Sir,

The War on Rats.

In order to obtain accurate information regarding the nature and extent of the damage done by rats within the United Kingdom my Committee have prepared a schedule of questions which they desire to place into the hands of all those who are in a position, from their own experience, to give valuable information concerning temporary or permanent rat plagues in their districts, the damage inflicted by rats, the steps taken by them—individually or in co-operation with others—for preventing such damage, the means chosen for that purpose, and the results obtained.

As the only means of gathering important knowledge of that kind is by favour of the Press—short of undertaking the appalling task of sending the questions to every one likely to suffer, or to have suffered, loss through rats, that is, to every householder in the country—my Committee venture to hope that you will permit an appeal to your readers to support the Society by asking for a copy of the schedule and returning it with such information as they may be able to impart.

I enclose a copy of the schedule for your information, and

am, Sir,

your obedient servant,

A. E. MOORE,
Secretary.

(*Schedule*)

TELEGRAMS: "NIMREV, LONDON." TELEPHONE NO. 43 PADDINGTON.

THE INCORPORATED SOCIETY FOR THE DESTRUCTION OF VERMIN.

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95, WIGMORE STREET,
LONDON, W.

Date ....

County of ....

Town (Village) of ....

.... Street

Number of Inhabitants ....

1. Name ....

   (Profession, Trade, etc.) ....

2. Description of place under report (state whether estate, farm, warehouse, factory, shop, etc.)

2A. Approximate area in square yards

2B. Number of persons living in the place

2C. Number of persons employed in the place

Total

2D. Describe the physical conditions of the district where your place is situated. Is there any factor favouring the existence of the rat plague?

3. How long has a rat plague existed, or how long have you been troubled with rats?

4. What are the particular kinds of loss inflicted by the rats?

A. Destruction of Food
B. ,, ,, Material
C. ,, ,, Animal Life

4A. Can you approximately state in £ s. d. the loss caused in any one year?

in 190 about £
in 190 about £
in 190 about £

5. What means have you employed for destroying the rats (rat catchers, traps, bacteriological preparations, what kind)?

5A. What was the cost per year? About £

5B. Were the rats exterminated?

5C. How long did your place remain free from rats after using such measures?

6. In using a poison or a bacteriological preparation, have you suffered any loss through domestic animals dying from eating poison or virus, or eating rats killed with poison or virus? Please state full particulars.

7. Have you made any systematic efforts to deal with the rat trouble, either alone or in co-operation with others?

7A. If so, what were the means employed?

7B. What was the form of co-operation (state whether co-operation between neighbours, the residents of a parish, or several parishes, or of a rat and sparrow club)?

8. Can you state the approximate result of such systematic efforts?

Number of rats killed in 190
,, ,, 190
,, ,, 190
,, ,, 190
,, ,, 190

Remarks:

9. Can you mention any particular incidents illustrating the power of rats for doing injury?

(*Form A*)

THE INCORPORATED SOCIETY FOR THE DESTRUCTION OF VERMIN,

LONDON.

GENERAL OBSERVATIONS.

1. In your experience, what is the best means on the market for destroying rats?
   (State what advantage it possesses in your opinion over other means).
2. Are you in favour of a proposal to multiply the number of the existing Rat and Sparrow Clubs, organising and co-ordinating them with similar agencies working for the extermination of rats?
3. If so, are you in favour of an annual grant being made by the State for that purpose, of say £10,000?

(*Form B*)

THE INCORPORATED SOCIETY FOR THE DESTRUCTION OF VERMIN,

LONDON.

STATISTICS.

1. Is it in your opinion reasonable to assume (for the purpose of estimating the loss caused by rats, by the destruction of private and public wealth) that there is *at least* one rat to each acre of the total acreage of the United Kingdom?
2. Is it in your opinion reasonable to assume that the number of rats present on farms, in hamlets and villages is *at least* equal to the number of inhabitants?
3. Considering the greater facilities provided for rats in the towns, as regards food and hiding places, is it reasonable to assume that there are also in towns as many rats as there are human beings?
4. Is it in your opinion reasonable to estimate the economic loss caused by rats (through eating and spoiling food and destroying material) at one farthing per rat per day?

SOME NOTES ON THE HAEMOGREGARINES PARASITIC IN SNAKES.

By C. CLIFFORD DOBELL,

Fellow of Trinity College, Cambridge; Balfour Student in the University.

(*From the Zoological Laboratory, Cambridge.*)

(Plate XX.)

THE object of this paper is to describe the protozoan parasites which I have encountered in the red blood corpuscles of three different snakes —*Boa constrictor*, *Python spilotes* and *Coluber quatuorlineatus*. All the Protozoa are members of the genus *Haemogregarina*.

Before recording my observations, I wish to make some general remarks about the blood parasites of snakes and the work which has already been done on them. The literature of the subject is so scattered and so curiously muddled at present, that I think no excuse is necessary for my attempting to summarise our present knowledge. Although I fully agree with Minchin's remark that, "it is not new species of haemogregarines that are needed, but rather new facts about old species[1]," nevertheless, I think these notes may be not wholly useless. It is my hope that they may be of service to other investigators who are in a position more favourable for working out the life-history of these very interesting parasites.

In the first place, it must be pointed out that we are at present quite ignorant as regards *the number of species* of haemogregarines which have been found in snakes. It is by no means certain that different hosts always harbour different species of *Haemogregarina*. So long as this remains a matter of uncertainty, there is bound to be difficulty in naming the parasites. In our present state of ignorance, by far the most suitable nomenclature—it seems to me—is that which simply refers the parasite to its host. For instance, the parasite of *Python* would be called *Haemogregarina pythonis*. This method—even should the name subsequently prove to be a synonym—can lead to very little confusion, and is at present of considerable utility. Laveran and

[1] Minchin, *Proc. Zool. Soc.* 1907.

others have already adopted this system. The nomenclature which calls upon the observer's friends to supply the specific names of the parasites may lead to much confusion, and is heartily to be condemned [e.g. the haemogregarines of two species of python are called "*H. pococki*" and "*H. shattocki*" by Sambon]. And although the rules of zoological nomenclature are purely arbitrary, it is advisable—even for medical men—to adhere to them for the present.

Some confusion has arisen through the names of the snakes examined by various observers. For example, Langmann in 1899 described haemogregarines under "the generic name of haemosporidia" (*sic*) in *Spilotes couperi.* In 1901, Lutz described "*Drepanidium serpentium*" in *Coluber corais.* In *C. corais* Sambon (1907) also described a similar parasite, and without taking any notice of previous workers, named it "*Haemogregarina rarefaciens.*" Now the snakes are all of the same species[1] in reality, and it is not improbable that the haemogregarines are also identical.

Another slight confusion in nomenclature has crept in through Börner (1901), who gave a table with a list of five haemogregarines and their hosts. Unfortunately, only one parasite is given to its proper host, the remaining four being distributed at random. This mistake has, apparently, been unwittingly copied by Minchin in his beautiful account of the Sporozoa (1903): so that three errors occur in his list of parasites and hosts.

At present nothing is known of *the way in which haemogregarines are transmitted* from snake to snake. Transmission may perhaps occur through the agency of an intermediate host (e.g. a tick)[2] or by way of the alimentary canal (as already described in similar frog parasites).

The *method of multiplication* appears to be by schizogony in the blood corpuscles—either whilst in the general circulation or in the viscera (spleen, lung, etc.).

No *sexual process* of any sort is known, and it is doubtful whether the forms described as males and females really are such. It will thus be seen that these animals afford a wide field for future work. It will, however, be needless for me to give further details regarding the various species. Instead, I have endeavoured to give as complete a bibliography as possible (p. 294) and have appended to my own observations a list of

[1] My authority for the names of the snakes has been throughout Boulenger's *Catalogue of Snakes in the British Museum.*

[2] Prowazek (1908) describes developmental stages (cysts etc.) in a pentastomid, *Porocephalus.* I regard these as exceedingly doubtful.

hosts and parasites (p. 292), which is as exhaustive as I have been able to make it. I may add that I have experienced considerable difficulty with the work of Sambon. As far as I am aware, Sambon has up to the present published merely a brief note in the *Lancet* (1907). Figures of his findings have been given, however, by Manson (1907), where many of the parasites are attributed to Sambon and Seligmann. Manson also gives some snake haemogregarines of which I can find no other mention. The information regarding them is, to say the least, scanty. In one place a new species is described from a "Mexican snake" under the name "*Haemogregarina brumpti* Sambon."

It may be remarked here that all the blood parasites of snakes—described under the names *Haemogregarina, Danilewskya, Drepanidium,* etc.—probably belong to the genus *Haemogregarina,* though perhaps in part also to the genus *Karyolysus.* The genus *Haemogregarina* was created by Danilewsky in 1885—*Danilewskya* Labbé 1894 being a synonym. If it be subsequently found that all the haemogregarines of snakes are really of the same species—which I think by no means impossible—then the correct name for the parasites is *Haemogregarina serpentium* Lutz.

I. *Haemogregarina* sp. from *Boa constrictor.*

(Plate XX, Figs. 1—13.)

This organism was obtained from a Brazilian *Boa constrictor* which had died of canker of the mouth. It is possibly identical with *Drepanidium serpentium* Lutz 1901 and *Haemogregarina terzii* Sambon 1907.

The parasites were very numerous, both in the general circulation and in the internal organs.

Small forms (Plate XX, Figs. 1, 2) were very uncommon. The commonest forms were those shown in Figs. 3—6. It will be seen that there are two distinct types of parasite—a long form with a recurved "tail" (Figs, 3, 4) and a stumpy form with rounded ends, and often with two vacuoles (Figs. 5, 6). The latter were not so frequently seen as the former.

Very large forms were fairly common, and many appeared to have arisen by the growth of the "tailed" forms (Figs. 7, 8). They attained a length of from 13μ to 15μ, and often appeared to have outgrown the corpuscles in which they had developed (Fig. 9).

In some of these large forms the nucleus had undergone partial fragmentation (Fig. 10), but I believe that this was the result of degeneration.

Certain of the parasites were distinguished by the possession of a very well-developed sheath (cytocyst), which stained a bright pink with Giemsa (Fig. 11). In other corpuscles I frequently found empty sheaths (Fig. 12), from which the parasites had evidently escaped. Free forms were also seen in the blood plasma (Fig. 13), and probably represent animals which have left their sheaths behind. The free forms attained a length of about 17μ.

I found doubly infected corpuscles on several occasions. No parasites were seen in the leucocytes.

From these facts it appears probable that the parasite enters a red blood corpuscle as a very small, falciform sporozoite or merozoite. In the corpuscle it grows into either a long or a stumpy organism—subsequently developing into a large fat form. It is possible that these two forms are sexually differentiated, and the large animals are possibly gametocytes, but this is merely conjectural.

At a certain period the parasite (? both forms) may envelope itself with a sheath (staining pink with Giemsa), from which it can subsequently issue. The significance of this is unknown.

I never found any forms which could be regarded as undergoing schizogony. Multiplication had probably ceased at this late stage of infection.

Details of structure will be readily seen by referring to the figures. It will be unnecessary for me to describe them more minutely here.

II. *HAEMOGREGARINA* sp. from *Python spilotes*.

(Plate XX, Figs. 14, 15.)

I obtained this organism from an Australian *Python*. It is perhaps the same as *Danilewskya pythonis* Billet 1895 (= *Haemogregarina pythonis* Labbé 1899) and *H. shattocki* Sambon 1907. Possibly *H. pococki* Sambon 1907 is another synonym. The haemogregarines of pythons have already been described by Billet 1895, Prowazek 1907, Sambon 1907 and Laveran 1908.

My organism is a typical haemogregarine (Figs. 14, 15) and shows no trace of the "blepharoplast" described by Prowazek. All the forms which I found were in approximately the same stage of development.

I have seen no differentiated "males" and "females" as described by Prowazek. Doubly infected corpuscles (Fig. 15) were several times seen.

Miss Robertson (1906) has described a haemogregarine-like organism from *Python* sp. under the name "*Trypanosoma pythonis.*" It has a well-marked kinetonucleus, but I think it is premature to place it in the genus *Trypanosoma* at present. The animal does not appear to be the same as mine.

III. *Haemogregarina* sp. from *Coluber quatuorlineatus.* (Plate XX, Fig. 16.)

Haemogregarines have not previously been described from this snake, though they have been found in allied species. I have only been able to examine a single snake, and that was only very slightly infected. A blood smear (2×1 in.) showed usually but a single parasite after careful searching. All the animals seen were at the same stage in development, and presented the appearance of an ordinary haemogregarine (Fig. 16). There was never any indication of a blepharoplast.

A single *Coluber melanoleucus* was examined with negative results.

In conclusion, I wish to thank Mr W. A. Harding for his kindness in allowing me to examine the snakes in which the parasites herein described were found.

LIST OF SNAKES INFECTED WITH HAEMOGREGARINES[1].

(In alphabetical order.)

Snake	Haemogregarine
Ancistrodon contortrix ...	"Haemosporidia" (Langmann 1899).
Ancistrodon piscivorus ...	"Haemosporidia" (Langmann, 1899): *Haemogregarina mocassini* (Laveran 1902).
Boa constrictor	"*Drepanidium serpentium*" (Lutz 1901): "*Haemogregarina terzii*" (Sambon 1907): *Haemogregarina* sp. (Dobell).
[*Bothrops*: see *Lachesis*.]	
Bungarus candidus ...	*Haemogregarina* sp. (Patton).
Bungarus fasciatus ...	"*Laverania*" *bungari* (Billet 1895) = *Haemogregarina bungari* (Labbé 1899).
[*Coluber aesculapii*: see *C. longissimus*.]	
Coluber corais ... ... (=*Spilotes couperi*)	"Haemosporidia" (Langmann 1899): "*Drepanidium serpentium*" (Lutz 1901): "*Haemogregarina rarefaciens*" (Sambon 1907).

[1] The haemogregarines referred to Patton, are quoted from a personal communication from Capt. W. S. Patton, I.M.S., who is recording his observations elsewhere.

Snake	Haemogregarine
Coluber longissimus (= *C. aesculapii*) ...	*Haemogregarina colubri* (Börner 1901): "Haemogregarine" (Finkelstein 1907).
Coluber quatuorlineatus ...	*Haemogregarina* sp. (Dobell).
Coluber sp.	"Haemogregarine" (Simond 1901).
Corallus cookii	"Haemosporidia" (Langmann 1899): "*Haemogregarina lühei*" (Sambon 1907—according to Manson)[1].
Coronella getula ... (= *Lampropeltis getulus*) ...	"Haemosporidia" (Langmann 1899): "*Haemogregarina wardi*" (Sambon 1907).
[*Crotalus adamanteus*: see *C. scatulatus*.]	
Crotalus confluentus ...	"Haemosporidia" (Langmann 1899): *Haemogregarina crotali* (Laveran 1902).
Crotalus scatulatus (= *Crotalus adamanteus*) ...	"Haemosporidia" (Langmann 1899).
Crotalus sp.	"*Drepanidium serpentium*" (Lutz 1901)[2].
Dendrophis pictus	*Haemogregarina* sp. (Patton).
Drymobius bifossatus ...	"*Drepanidium serpentium*" (Lutz 1901).
Dryophis mycterizans ...	*Haemogregarina* sp. (Patton).
Elaps fulvius	"Haemosporidia" (Langmann 1899).
Eryx conicus	"*Haemogregarina cantliei*" (Sambon 1907).
Eryx johnii	*Haemogregarina* sp. (Patton).
Eunectes murinus	"*Drepanidium serpentium*" (Lutz 1901).
[*Eutaenia*: see *Tropidonotus*.]	
Herpetodryas carinatus ...	"*Drepanidium serpentium*" (Lutz 1901).
Lachesis gramineus (= *Bothrops viridis*) ...	"Haemogregarine" (Simond 1901).
Lachesis mutus	"*Haemogregarina seligmanni*" (Sambon 1907).
Lachesis sp. (= *Bothrops* sp.)	"*Drepanidium serpentium*" (Lutz (1901).
[*Lampropeltis getulus*: see *Coronella getula*.]	
Macroprotodon cucullatus ...	"*Danilewskya*" *joannoni*: "*Drepanidium*" sp. (Hagenmüller 1898).
[*Morelia*: see *Python*.]	
Naia tripudians	*Haemogregarina najae* (Laveran 1902).
Naia tripudians var. *atra* ...	"Haemogregarine" (Simond 1901).
Naia sp.	"Haemogregarine" (Simond 1901).
Philodryas olfersii ...	"*Drepanidium serpentium*" (Lutz 1901).
Psammophis sibilans ...	"*Haemogregarina brendae*" (Sambon 1907).
Pseudaspis cana	"*Haemogregarina refringens*" (Sambon 1907).
Python molurus	"*Haemogregarina pococki*" (Sambon 1907).
Python reticulatus ...	"*Danilewskya*" *pythonis* (Billet 1895) = *Haemogregarina pythonis* (Labbé 1899).
Python spilotes ... (= *Morelia spilotes*) ...	"*Haemogregarina shattocki*" (Sambon 1907): *Haemogregarina* (Laveran 1908): *Haemogregarina* sp. (Dobell).
Python sp.	"*Trypanosoma*" *pythonis* (Robertson 1906).
Python sp.	*Haemogregarina pythonis* Billet (Prowazek 1907).
Rhadinaea merremii ...	"*Drepanidium serpentium*" (Lutz 1901).
[*Spilotes couperi*: see *Coluber corais*.]	

[1] The name is wrongly written "*Corellus cooki*."

[2] By a misprint the snake is named "*Curotalus*."

Snake		Haemogregarine
Spilotes pullatus ..	...	"*Drepanidium serpentium*" (Lutz 1901).
Tropidonotus fasciatus	...	"Haemosporidia" (Langmann 1899).
Tropidonotus ordinatus (=*Eutaenia sirtalis*)	...	"Haemosporidia" (Langmann 1899).
Tropidonotus piscator	...	"*Haemogregarina mirabilis*" (Castellani and Willey 1904).
Tropidonotus sauritus (=*Eutaenia saurita*)	...	"Haemosporidia" (Langmann 1899).
Tropidonotus stolatus	...	"*Danilewskya*" (Billet 1895) = *Haemogregarina* sp. (Labbé 1899).
Tropidonotus viperinus	...	*Haemogregarina viperini*[1] (Billet 1904).
Vipera aspis ...	...	"*Haemogregarina samboni*" (Giordano 1907—according to Manson).
Vipera russellii ...	...	*Haemogregarina* sp. (Patton).
Xenodon neuwiedii	...	"*Drepanidium serpentium*" (Lutz 1901).
Zamenis algirus ...	...	*Haemogregarina algiri* (Manceaux 1908).
Zamenis flagelliformis	...	"*Haemogregarina mansoni*" (Sambon 1907).
Zamenis hippocrepis	...	*Haemogregarina zamenis* (Laveran 1902; Manceaux 1908).
Zamenis mucosus ...	...	*Haemogregarina* sp. (Patton).

To this list may be added the following:

"*Drepanidium*," found in "a puff-adder from Gambia" (Dutton, Todd and Tobey 1907).

"*Haemogregarina brumpti*" from a "Mexican snake" (Sambon 1907—*fide* Manson).

"Haemogregarines" from "several varieties of green snakes and the cobra found on the island of Hong Kong" (Hunter 1908).

According to Labbé, haemogregarines were first seen in snakes by Pfeiffer.

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[1] Emended (!) by Manson to *H.* "*viperina.*"

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DESCRIPTION OF PLATE XX.

[All figures from permanent preparations stained by Giemsa's method. Drawings made under 3 mm. oil-imm. apochromatic obj. × comp. oc. 12 (Zeiss).]

Figs. 1–13. *Haemogregarina* sp. from *Boa constrictor.*

Figs. 1, 2. Young forms.

Figs. 3, 4. Larger forms, with slender recurved "tail."

Figs. 5, 6. Stumpy forms.

Figs. 7, 8, 9. Large forms.

Fig. 10. Large form with chromatin fragments in cytoplasm—probably degenerate.

Fig. 11. An organism lying inside its sheath (stained pink).

Fig. 12. Empty sheath in a corpuscle.

Fig. 13. Free form from blood plasma.

Figs. 14, 15. *Haemogregarina* sp. from *Python spilotes.*

Fig. 16. *Haemogregarina* sp. from *Coluber quatuorlineatus.*

THE TRANSMISSION OF *TRYPANOSOMA LEWISI* BY FLEAS AND LICE[1].

By GEORGE H. F. NUTTALL, F.R.S.

(a) *Transmission by fleas.*

The first experiments upon the transmission of *T. lewisi* by fleas were carried out by Rabinowitsch and Kempner (1899) who observed that three fresh rats placed with others harbouring trypanosomes in their blood subsequently (after 11—15 days) became infected. Fleas (species ?) were found on these rats. They next teazed the bodies of fleas taken from infected rats and inoculated them into fresh rats with the result that in five out of nine experiments the fresh rats became infected with *T. lewisi*. In one experiment they removed 20 fleas from infected rats and placed them on a clean animal with the result that the latter became infected after 2—3 weeks. They regarded this one experiment as conclusively proving that *T. lewisi* is transmitted by rat fleas.

Swingle (v. 1907, p. 119), who supposed he had observed a development of *T. lewisi* in rat fleas (species ?), cites some indirect evidence which indicates that fleas play an important part in the transmission of the trypanosome. Of 17 one-fourth grown rats examined in the autumn and winter not one harboured lice, fleas or trypanosomes. Of seven rats examined in the spring, at the same place, all harboured fleas and four harboured trypanosomes. Rats captured in other parts of Lincoln, Nebraska, harboured neither fleas, lice, nor trypanosomes. Fleas were found more frequently than lice on trypanosome-infected rats.

The single experiment by Rabinowitsch and Kempner has hitherto constituted the sole proof that fleas transmit *T. lewisi*. I desire to record confirmatory experiments which were carried out with every precaution so as to exclude any other mode of infection.

[1] Read before the Cambridge Philosophical Society, 23 Nov. 1908. The third and fourth experiments with fleas have been added.

Exp. I. Three fleas (*Ceratophyllus fasciatus*) were removed from a wild rat (*Mus decumanus*) which was infected with *T. lewisi.* The fleas were immediately placed upon a white rat whose blood had been previously examined at intervals with negative results for about a month. The rat remained isolated. Daily examination of the rat's blood proved negative until the 7th day when *T. lewisi* was found.

Exp. II. Four days after the trypanosomes had first been found in the blood of the rat in Experiment I, the animal was killed and one flea was recovered. This flea was placed on another tame rat, but in this case no infection followed. (Experiments I and II were carried out in March, 1908.)

Exp. III. In this experiment[1] the tame rat upon which the fleas (*Ctenopthalmus* [*Typhlopsylla*] *agyrtes* [Heller])[2] were placed was rigorously isolated in a flea-proof cage constructed after the pattern of those used by the Indian Plague Commission for their rat and flea experiments (see *Journ. of Hygiene,* 1906, Vol. VI. p. 435, Plate IV). The object of using this apparatus was to raise rat fleas in the laboratory. Eleven days (24. xi. to 4. xii. 1908) elapsed before trypanosomes appeared in the rat's blood :

Day 1. Blood examination of rat negative. Ten fleas were placed upon the rat, the fleas having been removed immediately before from a wild rat that was heavily infected with *T. lewisi.*

Day 3. Blood examination negative.

Day 4. Blood examination negative. Eight fleas were placed on the rat, the fleas having been removed immediately before from a wild rat which showed *T. lewisi* in its blood two days before it was killed and the fleas removed; no trypanosomes could be found microscopically on the day when the fleas were collected.

Days 5—10. Blood examination negative. On the 10th day, one flea from a wild rat infected with trypanosomes was transferred directly to the tame rat.

Day 11. Blood examination positive: 1 *T. lewisi* found in a fresh blood film.

[1] This experiment was carried out in conjunction with Messrs Patton and Strickland.

[2] Some of the fleas were kindly determined for me by the Hon. N. C. Rothschild, who states that *Ctenopthalmus agyrtes* has not apparently been found before on *Mus decumanus.* All the rats harbouring *C. agyrtes* were captured in one spot (Cherryhinton Brook, Cambridge). It is possible that one or two *C. fasciatus* may have been amongst the fleas put on this rat, since out of a batch of 18 fleas collected from rats from this locality one specimen belonged to this species. In Exp. IV the living fleas were determined by me before being placed on the white rat.

Day 12. Blood examination positive: 3 *T. lewisi* found in a fresh blood film.

Exp. IV. Three fleas (*Ctenopthalmus agyrtes*) were removed from a heavily infected wild rat and immediately placed upon a clean white rat in a flea-proof cage. After 10 days (8—18. xii. 1908) many trypanosomes were detected in the white rat's blood.

In Experiment II a single flea failed to transmit *T. lewisi.* In Experiments I and IV three fleas transmitted the trypanosome. In Experiment III it is reasonable to suppose that the trypanosome infection was due to the first batch of ten fleas which was placed on the experimental animal. The ease with which infection took place through the agency of fleas suggests that they are probably the chief transmitters of the trypanosomes. An extensive series of observations which we are at present making will however determine the relative importance of fleas and lice in the transmission of *T. lewisi.*

(*b*) *Transmission by lice.*

In four experiments carried out by Rabinowitsch and Kempner (1899) lice (species?) were removed from rats harbouring *T. lewisi* in their blood. The lice were dissected and inoculated into clean rats, but the rats did not become infected.

MacNeal and Novy (vi. 1903, p. 560) observed living *T. lewisi* in the stomach of lice (species?), a statement which is repeated by MacNeal (xi. 1904, p. 520) who reports that in one experiment "several such lice" were transferred to a fresh rat (4. ii. 1904) with a positive result in that *T. lewisi* appeared in its blood after 14 days and persisted therein for five weeks. MacNeal was unable to observe any development of *T. lewisi* in the rat-lice although he frequently observed the trypanosomes in them. He concludes therefore that "the louse merely carries the protozoon from one animal to the other."

On the other hand Prowazek (1905, p. 365) described what he took to be the development of *T. lewisi* in the rat louse (*Haematopinus spinulosus* Burmeister), but he failed to transmit the trypanosome from rat to rat by means of the lice. Nevertheless he concluded that these lice are certainly capable of transmitting the trypanosome. He apparently based this statement solely on the supposed development of the trypanosome in the rat louse.

In this connection I have also a positive experiment to record: numerous lice (*H. spinulosus*) were removed from infected rats. Great care was taken to secure their not being injured. The lice were then transferred to clean white rats, being placed close to the skin and covered by smoothing back the rats' hair over them. The lice promptly attached themselves to the hairs of the new host.

Exp. I. 20. iii. 1908. Many lice were removed from a wild rat (*Mus decumanus*) in whose blood *T. lewisi* could *not* be found. The lice were transferred to a white rat (*A*) which had been infected by blood inoculation with *T. lewisi*, and showed trypanosomes in its blood on the day before the lice were applied.

24. iii. 30 lice were removed from rat *A* and placed on a clean white rat (*B*). Rat *A*'s blood showed many trypanosomes at the time when the lice were removed.

26. iii. 30 more lice were removed from rat *A* and placed on rat *B*. Rat *A*'s blood contained many trypanosomes.

3. iv. 1908. *T. lewisi* found in rat *B*'s blood for the first time.

A second experiment carried out with fewer lice gave a negative result.

Exp. II. 3. iii. 1908. Ten lice were removed from a wild rat (*A*) infected with *T. lewisi* and placed on a clean white rat *B*.

10. iii. Four lice were similarly removed from another infected wild rat (*A*) and placed on white rat *B*.

14—24. iii. 1908, and subsequently, rat *B*'s blood gave negative results on examination.

The first experiment with lice demonstrates that 30—60 lice are capable of transmitting *T. lewisi* from diseased to healthy rats. In the second experiment 14 lice failed to transmit the trypanosomes.

I desire to note that a large number of lice have been examined in the course of the last year with a view to studying the development of the trypanosome in the louse as described by Prowazek. I have been aided in this work by Mr C. Strickland. We have hitherto been quite unable to trace any development of *T. lewisi* in *H. spinulosus*, and we have begun to seriously doubt that such a development actually occurs. At present we incline to the opinion that Prowazek was deceived by the presence of extraneous flagellates such as are known to exist in a number of blood sucking arthropods. Such flagellates have proved a fruitful source of error of recent years and in consequence great caution is required before reaching any final conclusions regarding what may

perhaps appear to be developmental stages of trypanosomes in invertebrate hosts[1].

Captain Patton, I.M.S., informs me that shortly after the publication of Prowazek's paper on the development of *T. lewisi* in *H. spinulosus*, he endeavoured to observe a similar development of *T. lewisi* in rat lice (*Haematopinus* sp.) in Madras. Beyond being able as we have done to recover the unchanged or degenerated trypanosomes he failed to find any of the stages described by Prowazek. Fleas (*Loemopsylla cheopis* Rothschild) also gave negative results on examination. Similar experiments with lice and fleas taken from the palm squirrel (*Funambulus palmarum*) infected with *Trypanosoma indicum* gave negative results.

In a preliminary note which has just been published by Manteufel (27. x. 1908) this author reports upon similar experiments. He states that he has been able to transmit *T. lewisi* by means of *H. spinulosus*, but gives no particulars regarding his investigations, reserving these for a paper which is to appear in the *Arb. a. d. Kaiserl. Gesundheitsamte.* He has in addition established the important fact that *H. spinulosus* is capable of transmitting *Spirochaeta recurrentis* from infected to healthy rats.

A series of 26 *Mus decumanus* which we carefully examined for *T. lewisi* and ectoparasites (30. x. to 3. xii. 1908) gave the following results:

		Ectoparasites present
3 harboured *T. lewisi*	... ...	Fleas only (15, 11, 1).
1 ,, ,,	... ...	Fleas (11) and lice (2).
1 ,, ,,	... ...	No fleas nor lice.
14 showed no trypanosomes	...	Lice on all of them.
1 ,, ,,	... ...	Flea (1) present.
6 ,, ,,	... ...	No ectoparasites.

The trypanosome rats all came from one locality, the uninfected rats came from three other localities. These observations again demonstrate the local character of the infection and support the view that fleas are the usual agents concerned in the transmission of the trypanosome. In a future paper we shall deal more fully with this aspect of the problem.

The experiments above recorded clearly demonstrate that T. lewisi is transmitted from rat to rat by means of Ceratophyllus fasciatus, Ctenopthalmus agyrtes and Haematopinus spinulosus.

[1] This aspect of the problem is discussed in another paper by Patton and Strickland in this number of *Parasitology*.

Since three distinct kinds of blood-sucking insects are capable of transmitting T. lewisi it appears doubtful that this flagellate is a parasite of the invertebrate "host" in the sense claimed by Prowazek and other investigators.

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ON THE PRESENCE OF AN ANTICOAGULIN IN THE SALIVARY GLANDS AND INTESTINES OF *ARGAS PERSICUS.*

By GEORGE H. F. NUTTALL, F.R.S.
and CYRIL STRICKLAND, B.A.

In the literature relating to the Ixodoidea there are a number of cases recorded of injurious effects following the bites of ticks. We do not refer to diseases like piroplasmosis and spirochaetosis which are known to be tick-transmitted, nor to other infective processes which may start at the seat of the tick's bite. The effects we refer to follow almost immediately upon the infliction of the bite and are distinctly toxic in character. These effects appear to have been more frequently observed following upon bites inflicted by species of *Argas* and *Ornithodoros*[1].

From the fact that strangers to a district are apt to suffer more severely than do the natives, it has been concluded that repeated tick-bites bring about a condition of immunity similar to that which has been observed in the case of mosquito bites. This appears to support the view that ticks give off something of the nature of a poison when inflicting their bites. On the other hand the toxic effects are by no means constant. In fact, in the case for instance of *A. reflexus*, the bites are only occasionally followed by immediate ill effects, and from this it has been argued that the persons who suffer possess a peculiar susceptibility or idiosyncrasy with regard to the poison.

It has not as yet been suggested that the differences in the after effects of the bite may also be due to differences in the substance injected into the wound by the tick. That this suggestion may however require consideration is indicated by an observation one of us has made with regard to a specimen of *O. coriaceus* (♀). The tick in question was captured in a wild part of Mexico, far removed from any human habitations. It is problematical from what source

[1] The literature on the subject has been fully dealt with elsewhere by one of us (1899, 1908), and need not be further considered here.

the tick had derived its food before it attacked the collector. The tick was removed almost immediately after it attached itself and it is doubtful if it actually drew any blood before it was forcibly detached. The bite produced a large violet ecchymosis within a few minutes and it took months for the wound to heal. On arrival in Cambridge this tick was allowed to bite a fowl with the result that a large haemorrhagic spot appeared about the wound, whilst the tick was feeding, and within half-an-hour or so an irregularly circular ecchymosis had formed measuring about two inches across. After the meal of fowl's blood had been digested it was again fed on a fowl on two occasions, but in neither case did any reaction take place around the seat of the puncture.

When fowls or other animals have been bitten by *A. persicus* or *O. moubata* in Cambridge, in the great majority of cases no reactions occurred about the minute punctures, but occasionally small ecchymotic areas appeared pointing, as in the case of *O. coriaceus* above cited, to a direct toxic action.

It is not known if ticks do or do not regurgitate material from their digestive tract whilst feeding. Should it be proved that regurgitation occurs, then an explanation of the occasional toxic effects might be found in the character of the regurgitated material which has been derived from a previous meal. On the other hand our experiments show that there may be considerable differences in the amount of anticoagulin which is present in the salivary glands of individual ticks. We may conclude from this that the amount of effective secretion injected into the wound may vary. But leaving hypothesis aside, it appeared desirable to learn something by experimental methods regarding the possible character of the salivary gland secretion.

A careful search of the literature has only brought to light one paper bearing upon the subject from the experimental standpoint. The investigations of Sabbatani (1898—1899), at Cagliari, demonstrated that the bodies of *Ixodes ricinus* (♂ and ♀) contain a substance which retards or prevents coagulation,—an anticoagulin. We have been able to go a step further by demonstrating the presence of anticoagulin in the salivary glands and intestines of *A. persicus*.

Sabbatani carried out his experiments as follows:

He removed replete female ticks from dogs, cut them in pieces with scissors, rubbed them up in a mortar together with salt solution, and filtered the fluid through muslin. Having added a known quantity of salt solution to a definite weight of ticks he utilized quantities of "tick solution" which corresponded to a given number of ticks

(i.e., 1 c.c. of solution corresponded to 1 tick). He found that 3—4 c.c. of solution prevented the coagulation of 20 c.c. of human and dog blood for 24 hours, that 8 c.c. of solution prevented the coagulation of 25 c.c. of ox and sheep blood, that 1 c.c. of solution prevented the coagulation of 5 c.c. of guinea-pig blood. When only 2 c.c. and 6 c.c. of solution were added to 25 c.c. of ox and sheep blood respectively, coagulation was markedly retarded. The anticoagulin also acted upon pig and frog blood.

When the solution was injected intravenously into dogs and their blood was sampled after intervals of 3—25—40 minutes, it was found that the blood samples did not coagulate or coagulated very slightly after 24 hours. The dose administered corresponded to about 1 gramme of tick per kilo of dog. The effect was less evident in the case of the cat, rabbit and guinea-pig. Lymph taken from the thoracic duct of dogs treated with the "tick solution" did not coagulate. A solution made from *male* ticks likewise exerted an anticoagulating action *in vivo*.

On heating the solution to boiling (100° C.), for 5—10 minutes, it no longer exerted an anticoagulating action. He extracted the active principle in the manner that Haycraft (1884)[1] did for "*hirudin*" in the case of leeches. He added absolute alcohol to the solution, collected the precipitate, dried it and redissolved it in salt solution. The extract prepared in this manner also contained anticoagulin.

Sabbatani found that intravenous injections of the solution produced grave effects in all the animals upon which he experimented: rapid and marked decrease in blood pressure; rapid heart-beat, soon followed by stoppage of the heart's action; respiration was slowed and then stopped. If the animals did not die whilst the injection was being practiced, they showed profound prostration, loss of reflexes, even complete paralysis. Moderate doses, administered to dogs and cats, caused diarrhoea, vomiting, loss of coordination, tremor, decreased blood pressure and rapid pulse, and the animals, after remaining feeble for hours, slowly recovered. Small doses exerted little or no effect. Dogs were very susceptible to the action of the tick extract, cats less so, whilst cattle and sheep were relatively resistant.

Blood corpuscles exposed to the action of tick extracts did not become haemolysed but they became crenated after 8—12 hours. The leucocytes appeared to be more resistant than the corpuscles.

The observations of Sabbatani have been quoted thus at length for

[1] *Arch. f. exper. Pathol. u. Pharmakol.* XVIII. p. 209.

the reason, already stated, that they represent the only scientific experiments bearing on the subject[1]. It will be noted that he extracted the anticoagulin from the tick as a whole and made no attempt to determine in which organs of the tick the antibody was present.

Methods.

The experiments here detailed were made with *A. persicus* obtained from South Africa through the courtesy of Mr C. P. Lounsbury, Government Entomologist, Cape Colony.

The salivary glands of the ticks were isolated by dissection under the microscope, they were rinsed off in ·8 % salt solution to remove extraneous impurities, and were then crushed between two clean slides in a minimal quantity of fluid. By raising the edge of one of the slides, the fluid gathered near one edge and practically the whole amount was then allowed to flow into a capillary tube. The tube was calibrated so as to measure about ·02 c.c. of fluid. When the gland emulsion had flowed in up to the mark, it was followed up by an equal quantity of blood taken as it flowed from a needle prick on the experimenter's finger. The gland emulsion and blood were then mixed up by alternately blowing out the fluid into a clean watchglass and then drawing it up into the capillary. The mixture was then drawn up into a clean capillary and allowed to stand for varying periods before it was examined. The examination consisted in blowing out the capillaries at stated periods to determine if coagulation had taken place or not. Control tubes were prepared in which an equal volume of salt solution and blood were mixed; in every case coagulation took place within a few minutes (7—8′) in the controls. Several preliminary experiments showed that salivary gland emulsions exerted a marked anticoagulating action. We record the following:

Experiments with Salivary Gland Emulsion and human blood.

Experiment I. One gland of *A. persicus* (♀, 6 mm. long) was emulsified in ·02 c.c. of salt solution and mixed with ·02 c.c. of blood. Result: Coagulation delayed for 30 minutes.

Experiment II. The glands of 4 *A. persicus* (♀, average length 7 mm.) were emulsified in ·05 c.c. of salt solution. The emulsion was

[1] We have been unable to consult the paper by Mosso (1899), but believe it contains only a report on Sabbatani's experiments. Grützner (1902) published a note with a suggestive title, but no contents either worth noting or original.

used concentrated and diluted to various degrees so that $\frac{1}{2}$ to $\frac{1}{16}$ of active principle was contained in the ·02 c.c. of saline which was mixed with an equal volume of blood. Result:

Dilution	1	prevented clotting completely.
,,	$\frac{1}{2}$	delayed clotting for 40 to 120 minutes.
,,	$\frac{1}{4}$	,, ,, ,, 30 minutes.
,,	$\frac{1}{8}$	,, ,, ,, 20 ,,
,,	$\frac{1}{16}$	,, ,, ,, 15 ,,

Experiment III. The glands of 4 *A. persicus* (♂, average length 6·5 mm.) were emulsified in ·05 c.c. of salt solution, the emulsion being used concentrated and diluted as in Experiment II. Result: Dilutions 1, $\frac{3}{4}$, $\frac{1}{2}$ all prevented clotting completely.

Experiment IV. The glands of 10 *A. persicus* (♂, 5·5 to 7·2 mm. long) were emulsified separately in ·02 c.c. of salt solution and each mixed with equal volumes of blood:

The glands of Tick No.	Delayed coagulation for	Size of Tick (length) in mm.
1	90 minutes	7·2
2	45 ,,	7
3	45 ,,	6·5
4	prevented completely	6·2
5	45 minutes	6
6	75 ,,	6
7	75 ,,	6
8	75 ,,	6
9	95 ,,	5·7
10	75 ,,	5·5

Experiment V. This experiment was made to determine if the emulsion of tick salivary gland exerted any deleterious effect on living leucocytes maintained at 35° C. The emulsion was mixed with a minute drop of human blood and the leucocytes were observed under the microscope.

(*a*) One gland was emulsified and mixed with blood.

Effect: Nil. Leucocytes actively motile after 2 hours.

(*b*) One gland in strong emulsion was mixed with blood.

Effect: Nil.

In none of the foregoing experiments was there any haemolysis observed even when gland emulsion was added in large amount to the blood.

Experiment with Rabbit's blood.

Experiment VI. A similar result was obtained with rabbit blood. The blood contained in the control tube clotted in 8 minutes, that in the tube containing salivary gland emulsion only clotted after 75 minutes.

From these experiments we conclude that the salivary glands of *A. persicus* contain a substance which prevents coagulation of the blood. The stronger the emulsion of gland the greater is the anticoagulant action. The amount of anticoagulin in the glands of different ticks varies considerably (Experiment II) and bears no relation to the size of ticks measuring 5·5 to 7·2 mm. in length. The amount of anticoagulin present in the glands will doubtless be found to depend upon the state of functional activity of the organs. The amount of anticoagulin present in the glands of a single tick is sufficient to prevent the coagulation of ·02 c.c. of human blood for 45 to 95 minutes, or even longer. The salivary gland emulsion exerts no inhibitory effect on the movements of leucocytes in extravascular blood and neither does it exert any haemolytic effect upon the red blood corpuscles.

Experiments made with emulsions of the intestines and human blood.

Having established the fact that anticoagulin is present in the salivary glands of *A. persicus*, it remained to be determined if anticoagulin is present in the intestines.

Experiment VII. The intestines of 3 *A. persicus* were dissected out, rinsed in salt solution, emulsified in a small drop of salt solution which was mixed in the proportion of 1 : 4 with blood.

Clotting was delayed for 2 hours. The corpuscles were not haemolysed. It is evident from this experiment that anticoagulin is present in the tick's intestines, and that haemolysin is absent.

Experiments in vivo.

It having been stated by Alt (1892, cited by Nuttall, 1899, p. 46) that an emulsion prepared by crushing 3 *Argas reflexus* produced toxic effects in a dog (comparable to those produced by small amounts of snake venom) on subcutaneous injection, it appeared desirable to control this observation. An emulsion of the body contents of 6

A. persicus was, therefore, prepared and injected subcutaneously into a mouse. The injection produced no noticeable effect. Salivary gland emulsion was similarly injected into a mouse (glands of 6 *Argas*), a cock (glands of 5 *Argas*) and a rabbit (glands of 5 *Argas*), but in no instance was the slightest local or general effect produced.

The effect of temperature upon the anticoagulin in the salivary glands of A. persicus.

In the case of the specific anticoagulin for rabbit's blood[1] Bordet and Gengou (1901) found that the antibody resists heating to 58·5° C. Sabbatani states that the anticoagulin present in *Ixodes ricinus* is destroyed by an exposure to 100° C. for 5—10 minutes.

To determine the temperature at which the anticoagulin in *A. persicus* is rendered inactive, we proceeded as follows: The glands of several ticks were dissected out and emulsified in salt solution, after which the emulsion was drawn up into capillary tubes. Some of the tubes were set aside as controls, others were heated to various temperatures for a period of 10 minutes. The capillaries which were heated were attached by elastic bands to a thermometer which was kept moving to and fro in a waterbath maintained at the temperature desired. After having been heated the emulsion was mixed with blood and tested in the usual way.

In a preliminary experiment, made with the glands of one tick, the unheated emulsion prevented coagulation for over $4\frac{3}{4}$ hours, whereas an emulsion heated to 55° C. was to some extent inactivated since complete coagulation of the blood with which it was mixed took place after 90 minutes.

In the next experiment an emulsion of the glands of several ticks was used. In the tube containing unheated emulsion coagulation commenced to take place after 61 minutes, whereas the heated tubes gave the following results:—

Heated to	Complete coagulation after
65°	23 minutes
70°	16 ,,
75°	12 ,,
80°	8 ,,

[1] Produced by injecting rabbit's blood into guinea-pigs. See Nuttall (1904), *Blood Immunity and Blood Relationship*, p. 17.

It follows that the anticoagulin is destroyed by an exposure for 10 minutes to a temperature of 80° C. and that its activity is reduced by lower temperatures, being considerably reduced even by a temperature of 55° C.

CONCLUSIONS.

1. There is clinical and experimental evidence that the bites of Argasidae may be occasionally followed by toxic effects which are either local or general in character.

2. This toxic effect may be due either to the peculiar susceptibility of the individual upon whom the bite has been inflicted or to the character of the substances injected into the wound by the tick in the act of biting. The cause of the toxic effect remains to be discovered.

3. The bodies of *Ixodes ricinus* contain substances which prevent the coagulation of the blood and cause toxic symptoms when injected into dogs. These substances do not cause haemolysis (Sabbatani).

4. The salivary glands and intestines of *A. persicus* contain anticoagulin but no haemolysin.

5. The amount of anticoagulin present in the salivary glands of *A. persicus* varies considerably. The amount contained in the glands of a single tick may delay the coagulation of ·02 c.c. of human blood for 45 to 95 minutes or indefinitely. The anticoagulin also acts on rabbit's blood.

6. The movements of human leucocytes remain unaffected by exposure to emulsions of the salivary glands of *A. persicus*.

7. Excepting the effects due to the presence of anticoagulins, it has not been established that the bodies or salivary glands of *A. persicus* contain toxic substances.

8. The anticoagulin in the salivary glands of *A. persicus* is destroyed by an exposure of 10 minutes to a temperature of 80° C. Its action is partially abolished by a similar exposure to 55° C.

Note whilst going through the press.

We have omitted to mention an interesting observation by Christophers (1906, p. 45) with regard to the fluid excreted from the "coxal glands" of *Ornithodoros savignyi*. Christophers casually remarks that " the fluid is slightly alkaline and prevents the coagulation of the

blood." He gives no further particulars. Colonel W. B. Leishman informs us that he has observed the same phenomenon with regard to the fluid excreted by *O. moubata*. He was led to suspect that the fluid might exert an anticoagulating effect because he observed that drops of blood, escaping from punctures made by *moubata*, failed to coagulate when they mixed with the fluid discharged by the ticks upon the skin of the host. Nuttall (1908, pp. 83, 97, 102) has observed the excretion of a similar fluid by *O. coriaceus* and *Argas persicus*.

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INOCULATION OF DOGS WITH THE PARASITE OF KALA AZAR (*HERPETOMONAS* [LEISHMANIA] *DONOVANI*) WITH SOME REMARKS ON THE GENUS *HERPETOMONAS*.

By Captain W. S. PATTON, I.M.S.

Since the discovery of the parasite of Kala Azar by Lt.-Col. Leishman, R.A.M.C., a number of investigators have endeavoured to reproduce the disease in the lower animals but without success. The recent discovery of Nicolle that dogs are susceptible to an allied human parasite (*Herpetomonas infantum*) has added a fresh stimulus to work along these lines, and in order to see whether the dog can be infected with *H. donovani* I carried out some inoculation experiments, which I propose recording in this paper.

Three young dogs were selected from a number of specimens brought to the King Institute; they had no ticks on them nor were they infected with *Piroplasma canis* and beyond being somewhat emaciated they were good specimens of bazaar dogs. Through the kindness of Lt.-Col. Robertson, I.M.S., I was able to obtain 10 c.c. of splenic blood from two typical cases of Kala Azar, and on examining some films it was found to be rich in parasites.

The dogs were inoculated as follows:

	Date	Weight	
Dog *A*.	9 June, 1908.	550 grms.	Inoculated with 2 c.c. intra-hepatic and 2 c.c. intra-peritoneal.
Dog *B*.	9 June, 1908.	306 grms.	Inoculated with 2 c.c. intra-hepatic and 2 c.c. intra-peritoneal.
Dog *C*.	9 June, 1908.	1124 grms.	Inoculated with 2 c.c. intra-peritoneal.

The inoculations were carried out with the greatest care, so that there could be no doubt that all the dogs received large numbers of the living parasites. The dogs were kept in clean iron cages free from ticks, their blood being examined at intervals with negative results. On June 19th the dogs were weighed and found to be as follows: Dog *A*, 820 grams, Dog *B*, 392 grams and Dog *C*, 1246 grams. On

June 30th Col. Robertson punctured the spleen of another case of Kala Azar and I obtained 6 c.c. of blood rich in parasites; Dogs *A* and *B* were each inoculated intra-peritoneally with 3 c.c. of this blood.

Dog *B* was killed on August 4th, when it weighed 514 grams. The following are the post mortem findings:

Spleen. Weight $12\frac{1}{2}$ grams, it appeared quite healthy; eight smears were made from different parts of the pulp and stained with Giemsa's stain. I was unable to find any parasites in them.

Liver. Weight 118 grams, there were no macroscopic appearances to be noted; eight smears were made from different situations, and were stained with Giemsa's stain. No parasites could be found.

The kidneys, lungs, intestines and peritoneum were quite healthy, and no parasites could be found in the bone marrow.

Dog *A* was killed on August 11th, when it weighed 1272 grams.

Spleen. Weight 26 grams, no macroscopic changes; 12 smears were made as above, but no parasites were found.

Liver. Weight 255 grams, healthy; eight smears were made, but they contained no parasites.

All the other organs were healthy.

Dog *C* is at present in the Institute at Madras.

From these experiments it would therefore appear that the dog is not susceptible to *Herpetomonas donovani*. The few dogs I have had the opportunity of examining in Madras have never harboured this parasite, and Christophers, who has had a much larger experience with these animals, does not record its occurrence.

Should it eventually prove to be the case that no animals are susceptible to the parasite of Kala Azar, it would further support the view that the Indian and Assam species is distinct from the Tunisian organism. I have been unable to collect any evidence in Madras that would support the view that dogs play any part in the transmission of Kala Azar. In Madras it is essentially a house disease and few of the people in Georgetown, where it is very prevalent, keep dogs.

The Genus Herpetomonas[1].

Believing as I do that the parasite of Kala Azar and its two allies belong to the genus *Herpetomonas*, I have for the last two years made a special study of this genus. These flagellates are parasitic in the

[1] This portion of the paper should be read in conjunction with my description of *Herpetomonas lygaei* in the *Archiv für Protistenkunde*, Vol. XIII. p. 1, 1908.

intestinal tracts of insects, non-blood-sucking and blood-sucking, and it should be clearly understood that those species occurring in the latter have no connection with any blood parasite, nor are they cultural trypanosomes; they have a characteristic developmental cycle which may be conveniently divided into three stages, *pre-flagellate, flagellate* and *post-flagellate.*

In their pre-flagellate stages they are round or oval bodies of varying size, and contain two characteristic chromatic bodies, the nucleus and blepharoplast. In this stage they multiply by simple longitudinal fission or by multiple segmentation, and are found in the midguts of their insect hosts, except in the case of the three human parasites. I have been able to show that this stage in a known species occurring in *Culex* mosquitoes is exactly similar to that of the human parasites. The flagellate stage is characterised by the formation of a *single* flagellum and the multiplication of the resulting flagellates by equal or unequal longitudinal division. The adult forms are long, spindle-shaped organisms, with a single flagellum, but no undulating membrane. This stage occurs in the mid- and hindgut of their hosts, but in the case of *Herpetomonas donovani* it takes place naturally in *Cimex rotundatus.* The post-flagellate stage is characterised by the massing together of the flagellates in the midgut, their shortening and rounding up, the resulting cysts are passed out in the faeces and are accidentally sucked up by fresh hosts. It is not known yet whether *H. donovani* undergoes this stage in the bed-bug.

It is important to remember that many of these *Herpetomonads* are indistinguishable in their *pre-flagellate stages,* and therefore if this stage alone is studied two distinct species may be classed as one. Further, I wish to point out here that a partial study of the stages of these flagellates is very apt to lead to confusion, so that what are true *Herpetomonads* may quite easily be mistaken for *Crithidia* or young *Trypanosomes*; a reference to recent literature will show that this has actually occurred in more than one instance.

As the life-cycles and general structure of the three human parasites are similar to those of well-known *Herpetomonads,* I see no reason for placing them in a distinct genus. The differences in their development, such as the formation of the flagellum, methods of division and the fact that their *pre-flagellate* stages are passed in man only justify their being regarded as specifically distinct from such species as *H. muscae domesticae, H. sarcophagae, H. culicis, H. lygaei,* and many others.

A TRYPANOSOME AND HAEMOGREGARINE OF A TROPICAL AMERICAN SNAKE.

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Plate XXI.

The trypanosome to be described in this paper was discovered in blood films taken from the snake *Erythrolamprus aesculapii* (Duméril and Bibron) of tropical America. For these films I am indebted to Dr Leiper. In addition to the trypanosome there was present in the blood a haemogregarine. Though haemogregarines are very common in snakes, especially in the Tropics, where nearly every snake examined is found to harbour these parasites, the reverse is the case with trypanosomes. Several observers have recorded the presence of trypanosomes in snakes but hitherto no one has given an accurate description of one of these either in the living or stained condition. Indeed our knowledge of the trypanosomes of the whole group of reptiles is very limited when compared with other groups of Vertebrata. On this account it seems of interest to place on record the characters of this trypanosome as it appears in the blood films mentioned above.

In the forthcoming third volume of *Reports of the Wellcome Research Laboratories*, Khartoum, I have described under the name of *Trypanosoma najae* a trypanosome of the spitting cobra *Naja nigricollis*. The trypanosome of the cobra was only met with in wet films and in spite of prolonged examination of many stained films not a single example of the stained trypanosome could be found.

In the present instance only three stained films (Leishman stain) of theb lood of *Erythrolamprus aesculapii* were available and the appearances of the trypanosomes in these films are as follows.

Two main types can be recognised and as with many other trypanosomes these may be distinguished as the wide and narrow forms. The

total length of both these varies from 30 to 35 μ. The width of the narrow forms is from a half to a third of that of the wide forms. Both are characterised by having the non-flagellar end much drawn out beyond the micronucleus or nucleus as the case may be. It may be so marked as to give the appearance of a whip-like process or flagellum.

The micronucleus is usually rod-shaped and prominent and is very uniform as regards its position in the body of the parasite. With reference to the nucleus its position varies but this is produced by the change in position of the nucleus rather than by any alteration in the situation of the micronucleus.

The flagellum arises from a point near the micronucleus and is never directly connected with this latter structure. It pursues a slightly wavy course along the border of the undulating membrane to the extremity of the body whence it is prolonged as a free flagellum for a distance of 5·5 to 7 μ. The flagellum is a delicate structure which does not stain very deeply and is developed to about the same extent as in *Trypanosoma lewisi.*

The nucleus consists of a roughly spherical group of fine chromatin granules and varies considerably in its position in the body of the parasite. It may be at the middle of the body (Fig. 4), or close to the micronucleus which, as pointed out above, varies very little in position. When in the latter situation, the nucleus may be on the flagellar side of the micronucleus as is usual in trypanosomes (Figs. 1 and 3), or it may be on the non-flagellar side (Fig. 5), as in the genus *Herpetomonas.* The occurrence of both forms in the blood of any animal is not common. Herpetomonas forms have been noted in the case of *Trypanosoma lewisi* and in *Trypanosoma theileri.* More recently such a condition has been described in *Trypanosoma wrublewskii.* In invertebrate hosts it is more common and Minchin has recently shown that a herpetomonas form occurs in the life cycle of the trypanosome of *Glossina palpalis.*

The flagellar extremity of the trypanosome is blunter than the very pointed non-flagellar end.

The protoplasm of the parasite stains blue and is free from granules. It shows a marked alveolar structure, while in the narrow forms it is as a rule darker than in the wider forms. Such a condition might lead one to suspect that the wide forms are merely flattened out narrow trypanosomes. From an examination of a large number of trypanosomes it is clear that this explanation is improbable. To some observers the wide and narrow forms would appear as female and male respectively but nothing is known of the history of this trypanosome and nothing in

support of this can be deduced from an examination of the stained blood films.

The body of the parasite is usually curved with the undulating membrane running on the convexity. This curve of the body probably represents the natural condition, for such a disposition of the body, though more marked, was a characteristic feature of *Trypanosoma najae* of the cobra.

Measurements of two of the trypanosomes were as follows:

	Form with nucleus and micronucleus separate	Form with nucleus and micronucleus adjacent
Non-flagellar extremity to micronucleus	9·8 μ	9·8 μ
Micronucleus to nucleus	4·9 μ	
Length of nucleus	2·1 μ	2·1 μ
Nucleus to flagellar extremity	11·2 μ	11·2 μ
Free flagellum	5·6 μ	7·0 μ
Total length of body	33·6 μ	30·1 μ
Width of body	4·2 μ	2·8 μ

The trypanosomes in the blood were fairly numerous: about one parasite to every 20 or 30 fields was the rule.

For this trypanosome which cannot be identified with any known species I propose the name *Trypanosoma erythrolampri* from its host *Erythrolamprus aesculapii.*

Haemogregarina sp.

It has been mentioned that a haemogregarine occurred with the trypanosome just described. This parasite differs very little from some of the haemogregarines already noted from other snakes. The red corpuscles are slightly increased in size and their nuclei displaced by the presence of the parasite. In other respects there is no recognisable change. The parasite usually occurs as an elongated slightly curved body with rounded ends measuring about 12·5 by 5 μ (Fig. 7). There is a delicate membrane or cyst enclosing the parasite which has its narrower end turned up. The cyst is less marked than it is in many haemogregarines. The protoplasm is vacuolated and the nucleus large and deeply staining. Some of the parasites are shorter and broader (Fig. 6) than the type just described but they show the same vacuolated protoplasm and deeply staining nucleus. The infection was a small one, there being many more trypanosomes in the films than haemogregarines.


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DESCRIPTION OF PLATE XXI.

Trypanosoma erythrolampri Wenyon, and *Haemogregarina* of *Erythrolamprus aesculapii* (Duméril and Bibron).

Fig. 1. Narrow trypanosome with nucleus adjacent to micronucleus.

Fig. 2. Wide trypanosome with nucleus at side of micronucleus.

Figs. 3, 4. Two wide forms with true trypanosome structure.

Fig. 5. Narrow form with nucleus and micronucleus as in *Herpetomonas.*

Figs. 6, 7. Two haemogregarines showing short and long form.

THE HAEMOGREGARINES OF MAMMALS AND REPTILES.

By Captain W. S. PATTON, M.B. Edin., I.M.S.

(*From the Quick Laboratory, Cambridge.*)

In two recent papers, Sambon and Seligmann (1907, 1908) have recorded some observations on the intracellular parasites of snakes, and have described no less than ten new species. The authors, in discussing the life histories of these parasites, have made the startling discovery that, "the life history of the *haemogregarines* like that of the *haemoprotozoa* is divided into two cycles: a schizogonic or 'vegetative' cycle spent in the blood of vertebrates and characterised by asexual multiplication, and a sporogonic or sexual cycle spent in the digestive organs of blood-sucking invertebrates and characterised by sexual reproduction." The authors then go on to speak quite familiarly of young *merozoites*, adult *schizonts*, adult *sporonts*, and so on.

My excuse for making some remarks on their findings is the fact that since November, 1905, up till July, 1908, I have worked with the following snakes infected with haemogregarines:

Bungarus coeruleus (*candidus*)	*Eryx johnii*
Vipera russellii	*Gonglyophis conicus*
Naja tripudians	*Dryophis mycterizans*
Python molurus	*Dendrophys pictus*
Zamenis mucosus	*Tropidonotus piscator*
	Tropidonotus stolatus

In all 250 snakes were at one time or another in the Laboratory at the King Institute, Madras, the majority of which harboured two species of *Aponomma*: *A. gervaisi* and another species not yet identified. Careful feeding experiments with the larvae, nymphs and adults of both these ticks were carried out in special receptacles, so that the conditions were very much as they occur in nature. In addition, I have had the

opportunity of studying no less than five haemogregarines in *Rana tigrina* and *Rana hexydactyla*, not only in the frogs but in the leech which transmits them. I have also studied the haemogregarine of *Emyda granosa* both in the tortoise and in the transmitting leech.

Lastly, I have had the unique opportunity of studying three mammalian *Leucocytozoa*: *L. funambuli*, *L. felis domestici* and *L. leporis*. With this large material at my disposal I have made an exhaustive effort to trace out the extracorporeal life histories of these intracellular parasites of mammals and reptiles, but in every case I have failed to find any developmental cycle in the corresponding blood-sucking invertebrates. In the case of the leech from *Emyda granosa*, Christophers once showed me some bodies which suggested developmental forms of the haemogregarine of the tortoise; I have examined these parasites in the leech, but can only come to the conclusion that they probably represent some stage in the life cycle of a *Coccidium* parasitic in the leech.

A few observations on *L. leporis* in the tick *Haemaphysalis flava* have suggested to me that the method of transmission of these parasites will eventually prove to be mechanical and that the characteristic vermicules,—*whose sex, by the way, I am at present unable to determine*,—free themselves in the intestinal tracts of the various invertebrate hosts and in some manner at present unknown make their way back to the biting parts. I have actual experimental evidence proving that the vermicules of *L. leporis* can remain alive in the alimentary tract of larvae and nymphs of *H. flava* for at least 15 days.

I am at a loss, therefore, to understand how Sambon and Seligmann have been able to observe adult *sporonts*, *schizonts*, etc., and how they are in a position to state these parasites have a *sporogonic* cycle in invertebrate hosts. It remains to be seen what observations they have made on the curious cycle found in the lungs and liver of snakes infected with haemogregarines. I have examined many examples of all the stages of this cycle not only in the stained condition but particularly in the fresh condition (for 12 hours) in the lung of *Zamenis mucosus*, and I have not been able to satisfy myself as to whether this cycle of multiplication is an asexual or sexual process; for this reason I could not definitely say what the different forms seen in the peripheral blood of all my snakes really represented. Yet I find Sambon and Seligmann call some of these *merozoites*, others adult *schizonts*, *sporonts*, etc. As far as I can gather they have principally studied these parasites in the peripheral blood of snakes, so that I cannot see their grounds

for these statements. Further, I would point out that I have examined haemogregarines in eight different genera of snakes and from a study of the parasites not only in the peripheral blood but also in the organs, I believe they belong to the same species. On the contrary Sambon and Seligmann make every haemogregarine they see in different species of snakes' peripheral blood a new species even in spite of the fact that they studied the parasites in this country when probably not a single snake had a tick on it. Without infecting a snake through the agency of the right tick and then studying the various forms of the parasites that appear in the blood and the organs of the snake, I do not see how it is possible to speak of the parasites in the peripheral blood as *schizonts, sporonts*, etc.

During the $2\frac{1}{2}$ years I have studied these intracellular parasites I have not felt myself justified in recording the results of my observations, as I considered my work would in no way advance our knowledge of these parasites, but after reading Sambon and Seligmann's papers I feel it is right I should record them, even though they are negative. Many keen observers in the tropics just beginning the study of these parasites and with excellent material at hand, on reading Sambon and Seligmann's papers, may come to the conclusion that there is nothing new to be learnt about them. I would like to advise them that this is not the case and that in my opinion the work of Sambon and Seligmann, instead of adding to our knowledge of these parasites, has increased the confusion already existing.

NOTE. While the above article was in the press a paper by Prowazek came to my notice. Prowazek speaks of free vermicules and cysts having a membrane with a double contour. The parasites he describes occurred in the Pentastome from a Python infected with *Haemogregarina pythonis*. He suggests that the cysts represent a further development of the haemogregarine. In November 1905, I examined a large number of Pentastomes (*Porocephalus pattoni*, Stephens) which are very common in the lungs of the rat snake *Zamenis mucosus* and found they were infected with what I then thought represented developmental forms of the haemogregarine of the snake. In addition to many free vermicules the Pentastomes contained cysts with a membrane having a double contour and containing smaller cysts full of spindle shaped bodies. I now know these cysts represent part of the cycle of a parasite peculiar to the Pentastome and have nothing to do with the haemogregarine of the snake.

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A CRITICAL REVIEW OF THE RELATION OF BLOOD-SUCKING INVERTEBRATES TO THE LIFE CYCLES OF THE TRYPANOSOMES OF VERTEBRATES, WITH A NOTE ON THE OCCURRENCE OF A SPECIES OF *CRITHIDIA, C. CTENOPTHALMI,* IN THE ALIMENTARY TRACT OF *CTENOPTHALMUS AGYRTES,* HELLER.

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It is now an established fact that a number of blood-sucking arthropods and leeches are infected with flagellate organisms belonging to the genera *Herpetomonas* and *Crithidia,* and we believe that in more than one instance, these natural flagellates have been described as developmental forms of various trypanosomes which might be ingested by these sanguivora. As the whole question of the transmission of trypanosomes is intimately connected with these so-called developmental forms in invertebrate hosts, we propose reviewing in detail this important subject before recording our observations on the flagellate of *Ctenopthalmus agyrtes.*

It will be remembered that the late Dr Schaudinn (1904) was the first to give a detailed description of the life-cycle of a trypanosome (*T. noctuae*) in a blood-sucking invertebrate, *Culex pipiens.* Although he referred to the similarity between his developmental forms in the mosquito and *Crithidia fasciculata* of Léger (1902), he made no reference to the possibility of his mosquitoes being infected with similar flagellates. Novy (1907) and his collaborators have clearly shown that both *Herpetomonas* and *Crithidia* may occur in the same mosquito, and one of us (1907) has traced the development of *Herpetomonas culicis* Novy from the larva of the mosquito through the nymph up to the adult insect, showing that even when mosquitoes are bred in the laboratory from larvae caught at large they may be infected with this flagellate.

Without actually repeating Schaudinn's experiments at Rovigno it is quite impossible to unravel his elaborate paper and to say whether he was dealing with a *Herpetomonas* or a *Crithidia* as well, not to mention the possible presence of *Spirochaeta culicis* in his mosquitoes[1].

Following Schaudinn's work, Prowazek (1904) claimed to have discovered the development of *T. lewisi* in the rat louse, *Haematopinus spinulosus*; but no subsequent observers, so far as we know, have found these developmental forms in rat lice. We have dissected and examined a large number of *Haematopinus spinulosus* from rats well infected with *T. lewisi*, but beyond finding unchanged[2] and degenerating trypanosomes we have never seen any of the forms described by Prowazek. One of us has also endeavoured to trace out the development of *T. lewisi* in rat lice, *Haematopinus* sp.?, in Madras with negative results. We are therefore forced to the conclusion that Prowazek has described part of the life-cycle of a natural flagellate, *Crithidia*, of *Haematopinus spinulosus*, and as such we believe it has no connection with *Trypanosoma lewisi*. We have examined Prowazek's figures and have no hesitation in saying they are exactly similar to appearances seen in other insects infected with *Crithidia*. For instance his figure 53 at once recalls an agglomerated mass of *Crithidia* such as one of us (1908) has recently depicted in the case of *Crithidia gerridis*; his figure 44 we would regard as a typical adult *Crithidia*, and his fig. 42, as a young form showing the development of the flagellum. It is important to note that Prowazek was unable to infect rats with lice which presumably had these developmental forms in them, nor does he mention the occurrence or not of these *Crithidia* in lice from rats uninfected with *T. lewisi*.

It is therefore of the utmost importance that this work of Prowazek should be confirmed or otherwise by those who have the opportunity of searching for this *Crithidia* of *Haematopinus spinulosus* in lice off rats from the same localities Prowazek obtained his 40 rats, viz. Berlin, Trieste and St Pelagio near Rovigno. We have searched so far in vain for this parasite in England. It is quite possible it is localised in its distribution.

The next important work on the development of Trypanosomes is that of Koch (1905), Gray and the late Captain Tulloch (1905). These

[1] In this connection see also Ross (1906, pp. 96, 101), Nuttall (1906, p. 109), Novy, MacNeal and Torrey (1906, p. 110).

[2] By this term we mean we have never seen parasites exhibiting changes such as occur in the *pre-flagellate* stages of *Herpetomonas* or *Crithidia*. We have certainly seen thin active trypanosomes which appear to have resulted from longitudinal division of ordinary forms.

observers stated they had discovered the development of *T. gambiense* and *T. brucei* in the *Glossinae*, but the later work of Novy (1906), Minchin, Gray and Tulloch (1906) has shown that tse-tse flies harbour natural flagellates in their alimentary tracts, and that these parasites are not connected with the pathogenic trypanosomes, *T. gambiense* and *T. brucei.* Minchin (1908), in his recent studies of these flagellates, made the important discovery that one of them, *T. grayi*, encysts in the rectum of *G. palpalis*; although at first agreeing with Novy that it is not connected with a vertebrate trypanosome, Minchin now regards it as an avian trypanosome. Minchin however does not attempt to explain how the cysts of *T. grayi* find their way back to the fly; it is presumed they are ingested by some bird, pass into its blood, and are again taken up by the fly when feeding on the bird. This extraordinary hypothesis appears to be based solely on the habits of the tse-tse fly as it is known to feed exclusively on the blood of vertebrates. In our opinion this fact does not exclude the possibility of these flies accidentally ingesting the cysts passed out in the faeces of other flies. At present very little is known of the habits of the *Glossinae*, and in order to understand how they may ingest these cysts it is important to find out whether they congregate at their breeding grounds, what they do shortly after hatching out, whether they fly off immediately to get a feed of blood or whether they remain sometime at their breeding grounds. These are certainly the occasions when they may accidentally ingest cysts passed out by other flies on leaves, twigs, etc. It is well known that blood-sucking species of Tabanids insert their proboscides into dew and other fluids on leaves, etc. when running about on these objects at their breeding grounds. It is important to examine tse-tse flies before they have had their first feed or even immediately after to see whether they contain any stages of *T. grayi* or other flagellates. In any case, we believe the cysts of *T. grayi* and their development into flagellates should be searched for in the alimentary tract of *G. palpalis.* The fact that Minchin (1908) and Stuhlmann (1907) found bacteria in the stomachs of tse-tse flies further confirms our view that these flies may become infected with other organisms, and we can find no proof either in Minchin's paper or in Stuhlmann's that these bacteria are derived from the blood these flies ingest or are inherited by them.

The single instance of a fly bred in captivity becoming infected with *T. grayi* after feeding on a fowl Minchin would regard as proof positive (*sic*) that this flagellate is an avian parasite. We can, however, find no mention of the fowl having trypanosomes in its blood, and Minchin

appears to have overlooked the possibility of the fly having parasites in its stomach before it fed on the fowl. Further, we find that this particular fly was bred from a pupa in August 1905, and that it was kept in a fly cage in which presumably other wild flies had been kept, so that it is not impossible for the fly to have ingested cysts of *T. grayi* passed out in the cage in the faeces of other flies, and as it was not killed till October 10th, 1905, there was ample time for this to take place and for the parasites to germinate; we think this is in the highest degree probable. It is fruitless to discuss this point any further; far more exact experiments and proofs are required before it can be accepted that *T. grayi* is an avian trypanosome.

Koch (1905) was the first to study the development of pathogenic trypanosomes in the *Glossinae, G. palpalis.* He states (1907) that he observed the development of *T. gambiense* in *Glossina fusca* and *Glossina tachinoides.* He differentiates the parasites into short and slender forms, zygotes with many nuclei which give rise to small forms. All Koch's attempts to inoculate animals with these forms have however proved negative. It is really difficult to see what connection these so-called developmental forms have with *T. gambiense.* Koch now regards the crocodile as a source of blood supply for tse-tse flies, and, in consequence of his statements it has been gratuitously assumed, more especially by the lay press, that the flies obtain the pathogenic trypanosomes from crocodiles. This assumption is entirely unjustified.

Turning now to Stuhlmann's (1907) recent work we find this observer dealt principally with *G. fusca* bred from pupae; the flies being fed for the first time on calves, sheep and dogs infected with *T. brucei.* After 2 to 4 days, 80—90 % of these flies developed a rich infection of flagellates in their alimentary tracts. Stuhlmann describes long forms which are found in the proventriculus and oesophagus but rarely in the proboscis; small forms which are almost exclusively found in the proboscis and seldom in the gut. He also describes amoeboid forms with or without flagella and regards them as resting parasites. Having studied these various forms Stuhlmann summarizes the development of *T. brucei* in *G. fusca* as follows: the cycle begins by a multiplication of indifferent forms in the intestine of the fly, the parasites then spread forward to the proventriculus where conjugation takes place, and as a result of this process small forms are produced which Stuhlmann believes are destined to pass into the vertebrate host. He was unable to find parasites in the end gut or any of the other organs. As a result of these observations on *G. fusca bred from pupae* and subsequently fed

on infected blood (*T. brucei*), Stuhlmann considers the above cycle represents the development of *Trypanosoma brucei.* It is, however, important to note that he was unable to infect animals with any of the forms he describes. Before criticising Stuhlmann's work it will be necessary to refer to two flagellates one of us has recently studied in Madras.

When endeavouring to follow the vermicules of *Leucocytozoon leporis* in the tick, *Haemaphysalis flava,* from *Lepus nigricollis,* the pre-flagellate stage of a flagellate was found in the gut diverticula of the larval tick; these forms corresponded exactly with similar stages of *Crithidia gerridis, Herpetomonas donovani* and *H. lygaei.* In fed larvae, kept for a few days, further development of these forms was observed, viz. the development of the flagellum, multiplication and formation of adult *Crithidia* and in the nymphs these flagellates were found in abundance. It was also discovered that only a certain percentage of the larvae from one adult was infected. Naturally the question immediately arose where had these flagellates come from? At first the only reply was, from the blood of the hare. Forty-two of these animals were examined when studying *L. leporis* and the blood of two on which a very large number of larvae was fed were frequently examined for months, both in the fresh and in the stained conditions, and, although many of the larvae had flagellates, no such parasites were ever seen in the blood of the hares. We are aware that a trypanosome (*T. cuniculi*) has been found by Jolyet and de Nabias (1891), Nicolle, Petrie (1904), Bosc (1904), and Bettencourt and França (1906), in *Lepus cuniculus,* and also that it is quite an easy matter to miss these parasites in the peripheral blood of animals. Yet, in spite of these facts, we believe that the flagellate found in the tick is in no way connected with a vertebrate trypanosome; the final proof of this will be dealt with elsewhere. Many hundreds of recently fed larvae of *Haemaphysalis flava* were examined, but we never found a trypanosome in any of them. As a result of these observations it was concluded that this *Crithidia* of *H. flava* is transmitted hereditarily. Swingle has informed one of us that he has worked out the mode of infection in the case of *Crithidia melophagia*; he finds that it is also transmitted hereditarily, thus confirming our observations on the hereditary transmission of *Crithidia.*

The other flagellate referred to above is found in the crop diverticula of a species of *Glossiphonia* sp.? parasitic on *Rana tigrina* in Madras. In addition to a number of haemogregarines, this frog is infected with two species of trypanosomes. After a long series of feeding experiments

with young leeches it was found that in one particular batch from one parent a large percentage developed an intense flagellate infection, while of another batch of young leeches fed on the same infected frog not a single one developed these flagellates, although exactly the same species of leech was used. It was eventually found that the explanation was quite simple: if the parent leech had flagellates in its alimentary tract a large percentage of its young also had them, whereas if the parent was not infected, its young also never developed flagellates. It was further impossible to trace any connection between these flagellates and the frog trypanosomes; we therefore believe they are true leech flagellates which are transmitted hereditarily. It is interesting to note that the flagellates developed in the leech from 2 to 4 days after feeding, exactly as in the case of the flagellates of *G. fusca*, and the small round forms of the leech flagellate were nearly always found in the anterior diverticula of the crop of the leech (cf. findings of Stuhlmann in *G. fusca*). So that in spite of the fact that Stuhlmann examined the other organs, presumably also the ovaries of *G. fusca*, we believe that the flagellate of this fly is transmitted hereditarily. We know it is exceedingly difficult to demonstrate the parasites in the eggs so that unless they were specially searched for at a particular stage they may readily be missed. In support of our view we would point out that Stuhlmann never found encysting forms similar to those of *T. grayi* in the rectum of *G. fusca*. Further Stuhlmann makes no reference to control experiments, that is to say feeding flies for the first time on animals known to be quite clean; we also note that no mention is made of the examination of the alimentary tracts of pupae of *G. fusca* descended from flies infected with flagellates. On examining Stuhlmann's figures (Plate X, figs. 151 to 158) we are unable to see what connection the flagellates represented have with *Trypanosoma brucei*. Figures 152 *a* to *g* are described as trypanosomes from a heavily infected fly; of these *a* is a good picture of a young dividing *Crithidia*, *b* another form showing the growth of the flagellum; the remaining figures *c* to *g* represent elongated *Crithidia* undergoing division; it is interesting to compare these figures with those of *Crithidia gerridis*. The proof as to how *T. brucei* comes to develop into these forms is in our opinion entirely wanting in Stuhlmann's work.

In a recent paper Keysselitz and Mayer (1908) claim to have fully confirmed Stuhlmann's observations on the development of *T. brucei* in *Glossina fusca*. These authors at the outset refer to Prowazek's work on the development of *T. lewisi* in *Haematopinus spinulosus*; we have

referred fully to this work above and have clearly shown that there is no evidence to support Prowazek's view of the flagellates he found in the rat louse. Keysselitz and Mayer, being unable to get sufficient material to breed out flies, *G. fusca*, studied the development of *T. brucei* in freshly caught insects. Judging from the contents of their intestines it would appear that all the flies had fed solely on mammalian blood, as no nucleated red blood corpuscles were seen. It seems to us to be very dangerous to presume that these caught flies had fed on animals infected with *T. brucei* alone, but this is apparently what Keysselitz and Mayer have done. 4·6 % of these flies were, according to them, infected with *T. brucei*, whereas 11·2 % which had been fed on healthy animals after being caught, were similarly infected; this difference is explained by saying that the parasites enter a swarming period and multiply after a meal when they are found between the "Epithel und Darmwand." The authors found the parasites in the proventriculus, proboscis, fore- and midgut, and in all cases in which they were in the proboscis they were also found in the other situations. All the flagellates we are told had the same general character, so that Keysselitz and Mayer regard them as representing part of the cycle of *T. brucei* as proved by Stuhlmann. We consider this proof is wanting for the reasons we have mentioned above.

In one hungry fly Keysselitz and Mayer saw many amoeboid non-flagellate forms as well as motile parasites between the "epithelium and intestinal wall." In the juice of the proboscis they found agglutinated stages of small trypanosomes which were attached in the proboscis on the oral side of the openings of the salivary glands; they conclude that they might be washed into the wound when the salivary glands empty themselves. The authors, however, do not say whether they tried to inoculate animals with these forms.

Keysselitz and Mayer fed their freshly caught flies on cattle which had become spontaneously infected with *T. brucei*; the blood of the cattle, they say, contained male and female forms of *T. brucei*, as described by Prowazek (1905). We are not convinced of the certainty of Prowazek's male, female and indifferent forms of *T. brucei*, and we have not seen any process of conjugation in this parasite. Even if it is admitted that there may be sexual dimorphism, Keysselitz and Mayer do not tell us how the zygote develops into the parasites they speak of in *G. fusca*. It is not at all clear to us why Keysselitz and Mayer fed their flies on infected animals, for they state that the flies had fed at large on animals and were infected with *T. brucei*. In these flies further development was

not seen, in spite of the fact, as the authors tell us, that they had fed on blood containing male and female forms of *T. brucei*. In another series of 96 freshly caught flies fed on the cattle, 10·4 % became subsequently infected with *T. brucei*, and it will be observed that this percentage corresponds to that of freshly caught flies, though, if they were feeding on suitable material containing male and female forms of *T. brucei*, it is only natural to expect more would become infected. We would not however have expected this, as we regard these flagellates of *G. fusca* as natural parasites. Keysselitz and Mayer also found that 11·2 % of freshly caught flies subsequently fed on healthy rabbits became infected, they do not explain how this took place. As their results are in direct opposition to Stuhlmann's observations on flies (*G. fusca*) bred from pupae when 80—90 % became infected, Keysselitz and Mayer explain this discrepancy by the fact that only 10 % of Stuhlmann's flies subsequently retained the infection. In our opinion the fact that in the majority of the flies the parasites tend to disappear from the alimentary tract, the longer the flies are kept, clearly suggests that the parasites have passed out of the digestive tube in their migration to the ovaries.

Keysselitz and Mayer conclude that *G. fusca* is only capable of being infected once in the course of its life, that is to say it is only once capable of offering conditions suitable for the further development of *T. brucei*, and that is when it takes its first feed of blood (from special animals) which contain male and female forms of *T. brucei*. All the trypanosomes which may be subsequently ingested die out, and this explains why other observers have failed to study the further development of *T. brucei* in freshly caught *Glossina fusca*. Why subsequently ingested male and female trypanosomes do not develop we must confess we do not understand.

Keysselitz and Mayer examined the "Geschlechtsproducte"[1] of four female *fusca*, and it would thus appear they at least suspect the possibility of the flagellates of the fly being transmitted hereditarily. Throughout Keysselitz and Mayer's paper we can find no reference to the possibility of these flagellates of *G. fusca* being anything more than harmless parasites of the fly and which have no connection with *T. brucei*. Although it is clear the only possible way they could be transmitted from fly to fly is by hereditary infection,—no encysted stages being found,—neither Stuhlmann nor Keysselitz and Mayer has carried out exhaustive feeding experiments to disprove this.

[1] By "Geschlechtsproducte," literally "sexual products," the authors doubtless mean the ova and the developing embryos.

We consider this is imperative and that the conclusions Keysselitz and Mayer have come to after using caught flies are erroneous and misleading.

Roubaud (1908) has recently devoted attention to what he considers to be a special development of pathogenic trypanosomes in the proboscis of *Glossina palpalis*. In two of these flies, *naturally* infected, he found flagellates fixed in tufts on the internal surface of the proboscis channel, walls of labrum and hypopharynx; the parasites were slender, measuring from 20—22 μ in length. The intestinal tracts of the flies also contained enormous numbers of flagellates, some of which were without flagella. Roubaud was unable to infect animals with any of these parasites. As a result of his observations he concludes that there are three methods of development of vertebrate trypanosomes in the *Glossinae*:

(1) Harmless culture of trypanosomes in the posterior portion of the midgut in the residuum of the digested blood. This culture disappears as soon as the flies are allowed to starve or when they feed again on blood.

(2) Special development of trypanosomes in the salivary fluid in the proboscis which is independent of (1) and is also as transitory; Roubaud considers this to be the important development in connection with the transmission of the pathogenic trypanosomes.

(3) An active multiplication in the intestine which may end in complete infection of the gut and proboscis, the parasites behaving like true parasites, and which until now have only been observed in cases of natural infection.

We can find nothing in Roubaud's work proving conclusively that these various developmental stages have come from vertebrate trypanosomes, and in spite of what he says we cannot see any difference between the forms he observed in the proboscis of *G. palpalis* and those described by Keysselitz and Mayer in *G. fusca*. We therefore can only consider these various developmental forms as representing stages in the life-cycles of one or more natural parasites of the fly which have no connection with any vertebrate trypanosome. The very fact that Roubaud was unable to infect animals with a pathogenic trypanosome by inoculating them with the forms he has seen in the proboscis of *G. palpalis* is, in our opinion, conclusive evidence that they are natural parasites of the fly. We have no difficulty in understanding that the conditions in nature are much more favourable for the infection of the flies with these natural flagellates. The three types of infection as

outlined by Roubaud in our opinion may quite well represent the development of two distinct flagellates of *G. palpalis*; one of these may be *grayi*, which we know encysts in this fly, while the other may be that known as *tullochi*, and it is possible the latter is transmitted hereditarily. Minchin's work on the encystment of *grayi* has not been followed up, on the contrary we find this flagellate is still regarded as a vertebrate trypanosome, and nothing whatever is known of the species *tullochi*.

Before concluding these remarks on the flagellates of tse-tse flies, we would like to urge on workers who have the opportunity of studying these parasites that it is of the utmost importance to ascertain how they are transmitted from one fly to another. We know the species *grayi* encysts in the rectum of *G. palpalis*; these cysts and their further development should next be looked for in the stomachs of this fly, either before it has first fed or soon after. The infection probably takes place in nature by the flies ingesting the cysts accidentally either before they fly off to get their first feed of blood or when they return to their breeding grounds between their several feeds. One experiment of Minchin's, quoted above, shows that it is possible to infect a clean fly in captivity. Freshly hatched out *G. palpalis* (after first ascertaining that they have not got a flagellate which is transmitted hereditarily), should be put in cages in which wild flies *G. palpalis* have been kept, some of these are sure to pass out the cysts of *T. grayi* in their faeces and it could then be seen whether the clean flies become infected.

In the case of *G. fusca*, and this applies also to all the other tse-tse flies, it could be readily demonstrated whether its flagellate is transmitted hereditarily by raising flies from infected parents and then feeding them on clean animals; pupae raised from infected flies should certainly be examined. It is important to keep in mind the possibility of any species of tse-tse fly being infected with two distinct flagellates, one of which may encyst in the rectum of its host and the other be transmitted hereditarily. One of us (1908) has recently made a suggestion which we have found of great use in studying these natural flagellates, and that is to divide their life-cycles into three stages, *pre-flagellate, flagellate* and *post-flagellate*; although these stages are not sharply separated from each other they are sufficiently distinct to enable anyone to follow the various forms as they occur in the flies. We believe that until these flagellates are completely studied, apart from any possible developmental form of trypanosomes, the important

problem in connection with the transmission of the pathogenic trypanosomes of Africa will not be completely solved.

We now propose dealing shortly with the question of the development of trypanosomes of fishes, frogs, eels and leeches. It has been accepted as an incontestable fact that the trypanosomes, mentioned above, undergo developmental changes in leeches. The first important work on this point is that of Keysselitz (1906) who claims to have followed the development of *Trypanoplasma borreli* in *Piscicola geometra.* This investigator, recognising the fact that leeches caught at large harboured flagellates, raised them from the egg, and on feeding them on fish infected with *Trypanoplasma borreli,* he observed certain developmental changes. He however failed to infect fish either by placing leeches on them or by injecting them with the gut-contents of infected leeches. We have pointed out above that in the case of a frog leech it was found that it harboured a flagellate which was transmitted hereditarily, and that it was not possible to trace any connection between this flagellate and frog trypanosomes. In the case of this particular leech it is not possible to exclude its natural flagellates merely by raising leeches at random; it is necessary first to make certain that the parent leech has no flagellates. Keysselitz makes no mention of this, and without the rigid exclusion of a natural flagellate we cannot accept this author's developmental cycle of *Trypanoplasma* as being free from error. It is also not at all clear how *Trypanoplasma borreli* passes into the developmental forms described by Keysselitz; in fact, this is exactly similar to what we have remarked in the case of the developmental forms of trypanosomes in tse-tse flies, lice, etc. Léger (1904) has also described the development of trypanosomes and trypanoplasms in leeches, and Brumpt (1904–1908) has extended this work, describing the development of trypanosomes of fresh water fishes, eels and frogs in various leeches. He also discovered that some of these leech flagellates, particularly those found in leeches from frogs, are transmitted to the progeny of the leech. França (1907) has also recently described the development of a frog trypanosome in a leech. In every instance, and we have looked carefully through the literature, these authors never mention the possibility of these flagellates being *harmless parasites of leeches.* It would appear that in each case leeches are fed on frogs etc. infected with trypanosomes; flagellates are later found in their crop diverticula and intestines and these are then described as developmental forms of the trypanosomes. In each particular leech the transition from the vertebrate trypanosome to the so-called

Crithidia-like or *Herpetomonad* form is exceedingly vague and abrupt, and it should be noted that no sexual cycle appears necessary in these trypanosomes. We are therefore unable to accept these authors' results and consider all their experiments are contaminated with possible natural flagellates we have referred to above.

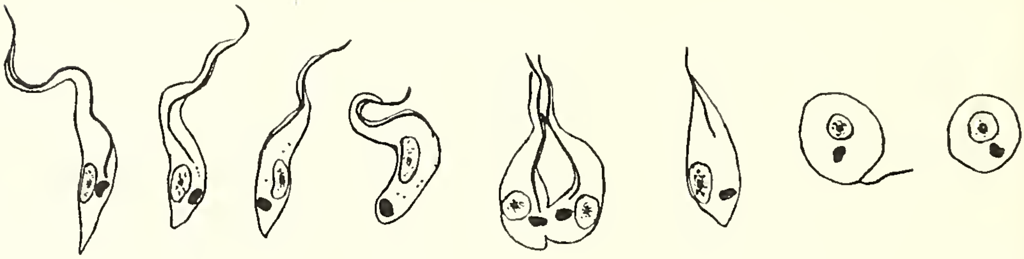
In *all* the instances in which vertebrate trypanosomes have been described as undergoing developmental changes[1] in invertebrate hosts, the presence or otherwise of natural flagellates has been entirely overlooked, and the work that is already recorded is of very little value. These natural flagellates are not "*cultural forms of trypanosomes*" but are true parasites of invertebrates. They have been found in *lice, fleas, mosquitoes, ticks, biting and non-biting flies, bugs* and in *leeches,* and we now propose recording our observations on a flagellate one of us found in the alimentary tract of *Ctenopthalmus agyrtes.* This flea, the Hon. C. N. Rothschild informs Professor Nuttall, has not been previously recorded from the rat, *Mus decumanus,* but that it is very common on mice, moles and shrews. We took a large number, as many as 16 off one rat, from a batch of animals caught at a particular locality in Cambridge. A large percentage of the rats were infected with *T. lewisi.*

Crithidia ctenopthalmi, n. sp.

We have examined the alimentary tracts of 25 fleas, and found two of them infected with this parasite; one of the fleas was from a rat infected with *T. lewisi* while the other flea came from a rat in whose blood we could not find any trypanosomes. The alimentary tracts of both the fleas, on being dissected out, were examined in the fresh condition; the midgut of the first contained fresh blood but no parasites could be seen moving in any part of it. The whole alimentary tract was ruptured and the contents smeared out and stained with Giemsa's stain; a number of adult flagellates and encysting forms were found in it. The midgut of the other flea contained digested blood alone, no parasites were seen in it but, on carefully examining the contents of the rectum, motile flagellates as well as round motionless forms were readily seen. The rectum was isolated and its contents smeared out and stained with Giemsa's stain. In this preparation it was possible to study the *post-flagellate* stage of this *Crithidia.* One of us (1908) has

[1] We do not deny the possibility of trypanosomes undergoing simple multiplication by longitudinal division; Minchin (1908) has clearly demonstrated this in the case of *T. gambiense* in *G. palpalis.*

pointed out that this stage is characterised by the flagellates collecting in the hindgut and rectum of their host, where they shorten, divide longitudinally, the flagella on degenerating are shed and the parasites finally round up into cysts.



Figs. 1—8. (×2900.)

In the film made from the contents of the rectum of the infected flea, noted above, we were able to recognise every stage from the adult flagellate up to the formation of the cysts. Fig. 1 represents one of the adult flagellates as yet unchanged, and it will be seen that its anterior end is prolonged along the flagellum, a short portion of which is free. The blepharoplast is a large rod-shaped structure, and in the majority of these unchanged flagellates, is seen lying either alongside the nucleus or just anterior to it. The flagellum is a single stout filament and arises in close proximity to the blepharoplast. In our preparation we have not seen any basal granule. On looking at a series of these flagellates we were struck by the fact that the blepharoplasts appear to migrate towards the posterior end so that these parasites come to look very like trypanosomes; we have depicted some of these appearances in figs. 2, 3 and 4. The explanation of this migration appears to us to be quite simple; in many of these forms it is seen that, though the flagellum is quite clearly stained at its free end, it is indistinct and hardly visible towards the blepharoplast and in some specimens this portion has quite disappeared (figs. 2, 3 and 4). We believe, therefore, that at this stage as the flagellum begins to degenerate the blepharoplast is freed and tends to pass towards the posterior end of the cell. We wish to draw attention to this peculiar appearance because it may, if studied alone, be quite well confused with a trypanosome. We have seen similar appearances in some *Herpetomonads* where the blepharoplast comes to lie behind the nucleus. At the same time as these changes are taking place, the anterior ends of the parasites become shortened

and fig. 4 shows an early stage in this process, while fig. 6 represents a more advanced stage, the anterior end having rounded up. One of us has figured and described somewhat similar changes in the post-flagellate stage of *Crithidia gerridis.*

It is quite common to find these encysting flagellates in all stages of division, and fig. 5 shows two very nearly separated. After the anterior end is drawn up completely the flagellum is shed and in some of the parasites they are seen as tags attached to the bodies of the parasites; we have depicted this appearance in fig. 7. In fig. 8 we show the final stage of encystment; these bodies are round or oval and contain a nucleus and blepharoplast. There were only a few of these forms in the preparations, the majority of the parasites were still flagellates. In other species we have found these cysts in enormous numbers in the rectum and hindgut of their hosts. This, then, is a mere outline of a part of the life-cycle of this *Crithidia*; we do not pretend that our observations even on this stage are complete. It would be necessary to examine the parasites in the fresh condition and watch these changes taking place. Unfortunately our material has been limited so that we are not able at present to undertake an extensive study of the parasite. It will be seen from our description and figures that this flagellate corresponds exactly with our definition (see below) of the genus *Crithidia.* In its adult flagellate stage the anterior end is drawn out, and it has a rudimentary undulating membrane; we propose therefore naming it *Crithidia ctenopthalmi.* It is important to consider how the fleas become infected, and we need hardly say that this flagellate is in no way connected with *T. lewisi.* We have examined a number of fleas (*C. agyrtes*) from rats heavily infected with *T. lewisi* but have never seen any developmental changes similar to those described by Prowazek in lice. The fact that we have seen the encysted stages of this parasite in the adult flea suggests that the cysts are passed out in the faeces of the flea and are ingested again either by the adult flea or its larva. It is obvious that the larva is much more likely to ingest the cysts and one of us has found a similar *Crithidia* in the larvae of *Ctenocephalus felis.* We are at present breeding fleas (*C. agyrtes*) and hope later, should we have sufficient material, to study this flagellate completely.

Flagellates appear to have been first recorded from fleas by Balfour (1906), who found them in the hindgut of *Loemopsylla cleopatrae,* Rothschild. This parasite, as far as we can judge from Balfour's figures, is a *Crithidia.* Swingle (1907) was the next to record the occurrence of flagellates in rat fleas (species not named) in Nebraska; he at first

mistook them for developmental forms of *T. lewisi*. We cannot say whether this parasite is a *Crithidia* or a *Herpetomonas*. Lastly, one of us, as mentioned above, found a *Crithidia* in the gut of the larva of *Ctenocephalus felis* in Madras. The larvae of this flea suck the blood of the cat and wherever a tame cat used to sit larvae gorged with blood were found in large numbers.

Concluding Remarks.

It is again necessary to make some remarks on the genus *Crithidia* of Léger as we find authors are still calling certain flagellates *Crithidia* which are clearly *Herpetomonads* and *vice versa*. This genus was created by Léger in 1902 for a flagellate he found in *Anopheles maculipennis*, and the name was based on what Léger considered to be the characteristic shape of this parasite, a short truncated (barley corn) organism. One of us has shown that the genus *Herpetomonas* also has a very similar stage and that the short truncated forms of *Crithidia fasciculata* are its young stages. We have also pointed out that by following Léger's description authors have placed a true *Herpetomonas* in the genus *Crithidia*, and *vice versa*. It is only necessary to refer to Léger's (1902) original description and figures of *Crithidia fasciculata*, when it will be seen that the adult flagellate of this parasite is exceedingly characteristic, and we have never seen any *Herpetomonas* like it. Owing to the fact that observers have so far only studied stages of these parasites, it can readily be understood how errors have arisen.

A great deal of the confusion regarding these flagellates is also undoubtedly due to Prowazek's erroneous view of the flagellar apparatus of *H. muscae domesticae*. One of us has shown clearly that, apart from the study of the *flagellate* stage of *Herpetomonas muscae domesticae*, by following the *pre-flagellate* stage up to the formation of the flagellum it can be demonstrated beyond any doubt that it has a single flagellum. Where then can the double flagellum come from? One of us has studied this flagellate for the last two years, and will shortly give a complete account of it where it will be shown that a fly may be found with almost all the flagellates showing the appearance of the double flagellum, and again in another fly the majority have a single flagellum. Finally, in following the *post-flagellate* stage of *H. muscae domesticae* the parasites have been observed to have *only* a single flagellum. The subject is still more confused by the recent introduction by Chatton, Alilaire (1908)

and Roubaud (1908) of the generic name *Leptomonas* of Kent. These observers would place all the *Herpetomonad*-like flagellates with one flagellum in this genus, while those with a double flagellum are relegated to the genus *Herpetomonas*, and Roubaud states he has observed the structure of *H. muscae domesticae* as described by Prowazek; so have we, but we are unable to give any other interpretation than that given above: we do not know on what Roubaud's statement is based. In order to make our first point clear as to how observers may be led astray by studying only one stage of a flagellate we will refer to another recent paper. Werner (1908) has described what he considers to be a *Crithidia* from the faeces of *Musca domestica*; unfortunately his photographs are so indistinct that it is not possible to make out clearly the structure of the parasite. If this parasite is a true *Crithidia* it is obvious that the author has again gone back to Léger's definition of the genus, and we consider this is a serious step backwards, and that instead of helping to a natural classification it will lead to greater confusion. We note that Werner's parasite was only found in the faeces; no mention is made as to whether the rectum and hindguts of the flies were examined for flagellates, and it is not stated whether the flies were infected with *H. muscae domesticae* as well. Werner refers to the similarity between his parasite and one recently described from *Culex pipiens* (*H. culicis*) by one of us. On referring to the figures of this *Herpetomonas* we find the only forms which appear to have a slight resemblance to Werner's parasite are those depicted in figs. 9, 10 *a*, 10 *b*. For the sake of clearness the various stages of this *Herpetomonas* were figured in a circle so that each stage could be readily followed. The figures to which Werner apparently refers clearly represent the young flagellates; it is our whole contention that a genus cannot be based on these immature forms. The adult flagellate of *H. culicis* is clearly depicted, and we fail to see what resemblance there is between it and Werner's parasite. One of us has seen parasites very like those described by Werner, and we know they represent the *post-flagellate* stages of *H. muscae domesticae.* In numerous living specimens we have seen the flagellates of the house fly collecting in masses in the rectum of the insect where the typical long forms shorten, divide and eventually round up, so that we would like to know what the adult flagellate of Werner's parasite is like. Remarking on the marked difference in size between his parasite and the flagellates of *H. muscae domesticae*, Werner believes it improbable that they could be in any way connected. We would draw attention to the still more marked difference in size between the cysts of *H. muscae domesticae* and the adult flagellate,

yet they undoubtedly belong to the same parasite. Again, we would refer to the striking difference in size between the cysts of *Herpetomonas lygaei* (3·5 μ to 4 μ) and the adult flagellate which may measure as much as 25 μ.

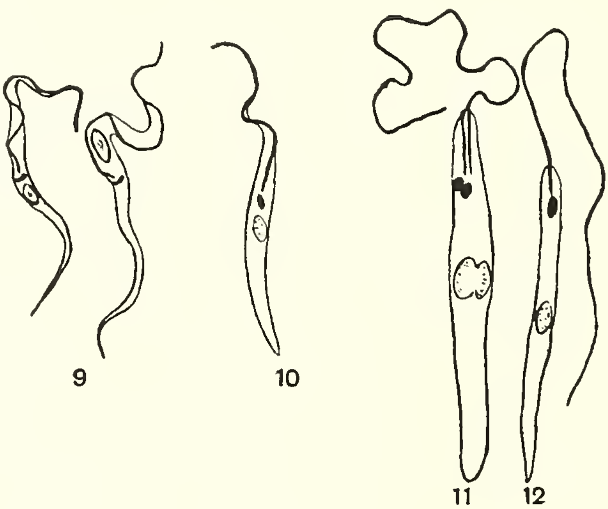
In order, therefore, to make our conception of the genus *Crithidia* quite clear we will define it as follows :—

Crithidia Léger, 1902 (emended by Patton, 1907).

Flagellates which in their adult stages have a fusiform body with a blepharoplast, usually a large rod-shaped structure, situated close to the nucleus either anterior or a little distance posterior; their anterior ends are attenuated and drawn out along the flagella to which they are attached by a narrow undulating membrane, which never has the characteristic folded appearance seen in adult flagellates of the genus Trypanosoma. Their posterior ends may be blunt or pointed. They have three characteristic stages in their life-cycles: pre-flagellate, *round or oval bodies with a nucleus and blepharoplast which multiply by simple fission;* flagellate *stage when they multiply by longitudinal division which may be either equal or unequal; in this stage they often exhibit marked polymorphism;* post-flagellate *stage when the flagellates shorten, divide and then encyst, some species (in ticks, leeches and Melophagus ovinus) pass this stage in the eggs of their hosts.*

Fig. 9 represents two adult flagellates of *Crithidia haemaphysalidis*, in one the blepharoplast is well behind the nucleus; note the pointed posterior ends. Fig. 10 is an adult flagellate of *Crithidia gerridis*.

These flagellates differ markedly from the genus *Herpetomonas*



Figs. 9—12.

which has a truncated anterior end, *single* flagellum and no undulating membrane. Figs. 11 and 12 are two flagellates of *Herpetomonas muscae domesticae* from the same fly; fig. 11 shows the appearance of the double flagellum, which we regard as the early stage in division, and fig. 12 represents an adult flagellate with a single flagellum.

The *Crithidia* are closely allied to the *Trypanosoma* in that they possess a rudimentary undulating membrane, and in some species, particularly those occurring in ticks (fig. 9), the blepharoplast passes behind the nucleus; many of these forms however represent early division stages (unequal) and in the typical adult forms the blepharoplast is never very far behind the nucleus.

These flagellates have up to the present only been found in the alimentary tracts and malpighian tubes of arthropods and leeches, and it is a remarkable fact that the majority of known species occur in blood-sucking invertebrates. In another paper in this *Journal* (p. 314), Wenyon records a very interesting flagellate from the blood of *Erythrolamprus aesculapii*; this parasite is the first flagellate we know from the blood of a vertebrate which at once suggests a *Crithidia* rather than a true trypanosome. A glance at Wenyon's figures will show that his flagellate differs considerably from the pathogenic trypanosomes of mammals. It is not at all unlike the flagellate from *Haemaphysalis flava*, which we believe is a *Crithidia*, both having attenuated posterior ends. It is impossible, however, to come to a definite conclusion regarding the exact position of the snake flagellate until more is known of its life-cycle.

We would pass the same remark on Chatton and Alilaire's (1908) parasite from the malpighian tubes of *Drosophila confusa* which they call *Trypanosoma drosophilae.* It is dangerous to form a definite opinion as to the biological position of these insect flagellates on morphological grounds alone and we cannot too strongly draw attention to the fact, *that a knowledge of the complete life-cycle of a protozoön is of the greatest value, for it is only then possible to form an adequate conception of any species and its relationships.* We have pointed out above that in a certain stage in its life-history a *Crithidia* may be very like a young trypanosome, e.g. a short form of *T. dimorphon.* We would also like to draw attention to the great tendency there is at the present time of theorising on the origin of the trypanosomes and allied flagellates. Prowazek (1904, 1905), Brumpt (1908), Minchin (1908), Woodcock (1906), and others have each advanced a particular hypothesis of their own, and now we find Chatton and Alilaire (1908)

bring forward another on the polyphyletic origin of the *Trypanosomatidae.* Is the evidence to hand sufficient to justify this hypothesis ?, we think not. Chatton and Alilaire have only observed one stage of *T. drosophilae,* and we are not even told whether this is the *adult* flagellate stage, so that it is difficult to accept the authors' contention that this parasite is a true trypanosome.

In the present state of our knowledge it is imperative that we should obtain accurate and detailed descriptions of the life-cycles of these flagellates of arthropods and leeches. It is also essential to determine the manner in which infection occurs. We may then be in a position to theorise on the structure and origin of the Trypanosomatidae and other allied forms.

In the meantime we propose placing the following species in the genus *Crithidia* :—

1. *Crithidia fasciculata* Léger, type species parasitic in the alimentary tract of *Anopheles maculipennis.* Only a part of the life-cycle of this parasite is known. Mezincesco (1908) in a recent paper refers to this flagellate as *Trypanosoma culicis* Novy (1907); *Crithidia fasciculata* of Novy, MacNeal and Torrey is obviously *Herpetomonas culicis.*

2. *Crithidia campanulata* Léger, parasitic in the alimentary tract of *Chironomus plumosus,* life-cycle not known.

3. *Crithidia minuta* Léger, parasitic in the intestine of *Tabanus tergestinus,* life-cycle not known.

4. *Crithidia subulata* Léger, parasitic in the alimentary tract of *Tabanus glaucopus* and *Hematopota italica,* life-cycle not known.

5. *Crithidia gerridis* Patton, parasitic in the alimentary tract of *Gerris fossarum,* life-cycle recently described (Patton).

6. *Crithidia tabani* n. sp. Patton, parasitic in the alimentary tract of *Tabanus hilarius,* life-cycle will shortly be described in *Archiv für Protistenkunde.*

7. *Crithidia grayi* Novy, parasitic in the alimentary tract of *Glossina palpalis* and possibly other *Glossinae,* life-cycle partly known (Minchin).

8. *Crithidia tullochi* Minchin, parasitic in the alimentary tract of *Glossina palpalis* and possibly other *Glossinae.*

9. *Crithidia* sp. ?, parasitic in the alimentary tracts of *Glossina fusca* and *Glossina morsitans,* part of the life-cycle described by Stuhlmann and Koch.

10. *Crithidia melophagia* Flu, parasitic in the alimentary tract of

Melophagus ovinus, life-cycle shortly to be described by Swingle, part has been described by Flu (1908).

11. *Crithidia christophersi* Novy, parasitic in the alimentary tract of *Rhipicephalus sanguineus.*

12. *Crithidia haemaphysalidis* n. sp. Patton, parasitic in the alimentary tract of *Haemaphysalis flava.*

13. *Crithidia robertsoni* n. sp. Patton, parasitic in the crop diverticula and intestine of *Pontobdella muricata,* part of life-cycle described in detail by Robertson (1907), method of infection not clear; it is believed by Brumpt and Robertson to be connected with *T. rajae,* this is however not proved.

14. *Crithidia ctenopthalmi* n. sp. Patton and Strickland, parasitic in the alimentary tract of *Ctenopthalmus agyrtes,* part of the life-cycle described. Other unnamed species have been found in *Loemopsylla cleopatrae* and *Ctenocephalus felis.*

15. *Crithidia haematopini* n. sp. Patton, parasitic in the alimentary tract of *Haematopinus spinulosus,* part of the life-cycle has been described by Prowazek.

It is impossible to include in this list doubtful species such as *T. drosophilae*; the flagellate found in the ovaries of *Chrysops dimidiatus* by Ziemann (1905) which may be but a stage of a parasitic flagellate and another found by Léger in *Nepa* and named *Otomonas tremula.*

It is also impossible to include many more which have been found in bugs by Donovan in Madras, by Léger, Brumpt, Leydig and others in leeches, and by Leydig in ticks; it is possible that some of the flagellates described by Léger as *Herpetomonas* may eventually have to be placed in the genus *Crithidia.*

In conclusion we wish to thank Professor Nuttall for his kindness in helping us to complete this work, and we hope that the critical remarks made in this paper may be of some use in guiding subsequent workers to a better understanding of these parasitic flagellates of arthropods and leeches.

APPENDIX BY CAPTAIN PATTON.

A recent paper by Mesnil and Brimont entitled "Sur un Hématozoaire nouveau (*Endotrypanum,* n. gen.) d'un Edenté de Guyane" (*Compt. Rend. Soc. Biolog.* T. LXV. No 35, 11th Dec. 1908, pp. 581–583, 7 figs.) calls for some remarks. The parasite here recorded was apparently found by Brimont in the red blood corpuscles of the common

Edentate, the two-toed sloth, *Cholaepus didactylus* (Linn.) in St Laurent du Maroni. The parasite measures from 8—11 μ in length and from 2·5—4 μ in breadth, it is rounded at one end and pointed at the other, it is not pigmented. When stained by Giemsa's stain it is seen to contain a circular nucleus and a well defined rod-shaped blepharoplast either lying beside the nucleus, anterior or posterior to it. The pointed end is described as the anterior and although in some of the parasites it is filamentous no flagellum could be demonstrated. The invaded corpuscles are not hypertrophied, nor do they contain any granules; they are simply deformed owing to the shape of the parasite. In the majority of the corpuscles only one parasite was found but in one there were two. Two elongated bodies, possibly parasites, were seen in a mononuclear leucocyte.

The authors remark that this intracellular parasite recalls the *Haemocytozoa*, for example the haemogregarines, in that it invades a red blood corpuscle, is elongated and not pigmented. They consider it constitutes an intermediate type between the *Trypanosomata* and the *Haemocytozoa*, like *Leishmania*, since it shows in the blood of a vertebrate host all the morphological characters of a true flagellate as *Leishmania* shows in cultures (*sic*) and in *Cimex rotundatus*. Mesnil and Brimont believe that this new parasite is more closely related to *Trypanosoma* than to *Leishmania*, and, as it invades the red blood corpuscles of a vertebrate, they have created for it a new genus, *Endotrypanum*.

From an examination of the authors' figures of the stained specimens it is clear that the parasite does not possess a flagellum; it therefore cannot have all the morphological characters of *Herpetomonas donovani* as seen in cultures and in *Cimex rotundatus*; I have clearly shown that the parasite of Kala Azar is a *Herpetomonas* and not a *Haemocytozoon*. It is however certain that the parasite of the sloth represents a stage in the life-cycle of a flagellate, probably a part of the *post flagellate* stage. The question naturally arises, how does it invade the red blood corpuscles? The shape of the parasite certainly suggests that it may penetrate them when possessing a flagellum which later disappears. No free forms, similar to those in the corpuscles, were seen. It is possible this intraglobular stage is a transitory one and that the parasite undergoes other changes in the organs of the sloth. The rounded binucleate stage should certainly be searched for in the leucocytes and endothelial cells as well as in the transmitting invertebrate.

The structure of this organism suggests that it is closely allied to the *Crithidia* as defined above. These flagellates have so far only been

found in invertebrates, and, as we now know, three Herpetomonads which pass a part of their life-cycles in a vertebrate, and a large number of trypanosomes which only live in the blood plasma of vertebrates, there seems no reason why the closely allied *Crithidia* should not be found also in the blood of vertebrates. *Crithidia* are common in blood-sucking invertebrates and we know of some that are well able to penetrate the ova of their hosts, so that there is no reason why they should not be able to penetrate red blood corpuscles. I am unable to follow the authors when they say that the discovery of this parasite supports Schaudinn's view on the phylogenetic relations of *Trypanosoma* and *Haemocytozoa*. There is no evidence to show that the parasite is a *Sporozoon*. The fact that it invades the red blood corpuscles of a vertebrate merely indicates that it is a highly specialised flagellate; cf. the mammalian leucocytozoa, *L. canis*, *L. funambuli*, etc. The occurrence of a flagellate in the corpuscles of a vertebrate further emphasizes the fact that we know very little about these parasitic flagellates and that without a complete knowledge of all the forms and their life-cycles it is futile to construct hypotheses. The discovery of further stages in the development of this new flagellate will be awaited with great interest.

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ON THE STRUCTURE OF THE SPIRACLES OF A TICK—*HAEMAPHYSALIS PUNCTATA*, Canestrini and Fanzago.

By G. H. F. NUTTALL, F.R.S., W. F. COOPER, B.A. and L. E. ROBINSON, A.R.C.Sc.

Plates XXII, XXIII.

The detailed structure of the spiracles in the Ixodoidea has hitherto received little or no attention at the hands of zoologists; at the same time, these organs are sufficiently extraordinary to make it a matter of surprise that, so far as our knowledge of the literature goes, not one of the numerous contributors to the subject of tick anatomy has found it worth while to undertake a complete description or to publish figures to illustrate it. Batelli (1891) gives a short account of the structure of the spiracle of a tick, presumably *Ixodes ricinus*, with a single figure, but with this exception we have been unable to find any further information on the subject.

The material for this communication has been accumulated in the course of our work on the anatomy of ticks, and in view of the interesting features presented by the spiracles, we have preferred to publish a separate account of these structures, although a more or less general description will appear later in the second part of our paper on the anatomy of *Haemaphysalis punctata*[1].

The spiracles are situated in the posterior half of the body, on the lateral margins, towards the ventral surface, immediately behind and a little external to the coxae of the fourth pair of legs. Each consists of a slightly elevated "plaque" with a well-defined margin. The dimensions of the spiracle show a considerable amount of variation; in the adult tick the length of the spiracle usually exceeds the breadth, in the nymph the reverse is the case. The following table gives the

[1] See this volume, pp. 152—181.

actual dimensions in both sexes and the nymphal stage, measured on ten specimens of each taken from a large number collected in Kent.

Dimensions of Spiracle in Haemaphysalis punctata.

(In microns.)

Male		Female		Nymph	
Length	Breadth	Length	Breadth	Length	Breadth
510	320	500	430	160	170
500	310	460	410	160	160
500	310	430	360	160	150
500	300	410	400	150	170
490	350	400	360	150	170
480	330	390	360	140	160
470	320	360	350	140	150
460	270	350	320	140	150
440	260	340	320	130	150
430	270	330	330	130	140

The contour of the spiracle differs in the three cases, although in the case of the male an approach in shape to the female type may be frequently observed. The spiracle of the female (Pl. XXII, fig. 1) is more or less angular in outline, this being due to a flattening of the contour of the lateral margin; that of the male has a more or less rectangular figure and becomes gradually narrower towards its posterior portion. The spiracle of the nymph is almost circular, with a slight rounded angle at the postero-lateral margin.

The surface of the spiracle is slightly concave with the exception of a small central area, the macula (Pl. XXII, figs. 1 and 2), dark in colour and slightly eccentric in position, situated a little towards the antero-mesial margin. The macula is elliptical in outline and placed obliquely with the major axis inclined at an angle of about 45° to the median axis of the body, the anterior end being directed outwards. The marginal portion of the spiracle is formed of dark-coloured chitin, and the area between this margin and the macula, of a pale greyish-yellow colour, is perforated by numerous regularly-distributed minute pores (*e.p.*); these are best seen by examination of uncleared specimens in reflected light. A limited number of coarser pores open on the marginal portions of the plate (*m.p.*). The macula of the spiracle of the female is cleft by a large slit-like opening which we have termed the ostium (Pl. XXII, figs. 1, 2 *os.*); the extremities of the latter are curved round in a crescentic manner with a convexity towards the antero-mesial margin of the spiracle. It is bounded on its external

side by a prominent raised lip (Pl. XXIII, figs. 5—7), which overhangs the opening and effects its closure when the lip is depressed by muscular action. The ostium does not appear in the male or nymph[1].

It is only when cleared and mounted specimens of the entire spiracle are examined that its complex structure becomes apparent. That which on examination *in situ* appears to be a simple sieve-like plate, resolves itself into a series of three superposed layers, each differing from the other two in structure. The superficial layer exhibits a regular reticulate pattern which, as will be seen later, is due to a thickening of the chitin on its under surface; the meshes of this reticulum are more or less circular, and each is perforated in its central portion by one of the minute pores (*e.p.*) already alluded to. It is almost impossible to define these pores in cleared entire preparations of the spiracle on account of their extremely thin margins being obscured by the underlying parts. Immediately beneath the superficial layer is a large air space, traversed by an arrangement of innumerable delicate chitinous rods or pedicels (*p.*), the slightly expanded bases of which arise from a thick basal layer of chitin, the latter forming the deepest layer and the most substantial portion of the spiracular plate. The basal layer, like the superficial layer, is porose, but its pores (*i.p.*) are not pervious to air, being occupied by protoplasmic extensions from the hypodermal cells underlying the spiracle. The internal pores coincide in position with the superficial pores and are those seen in ordinary cleared and mounted preparations. The intermediate or pedicellar layer is formed by the system of pedicels which support the superficial layer. The arrangement of the pedicels shows a regular system; this is readily seen in Pl. XXII, figs. 2 and 4. The pedicels are triangular in cross section and are slightly curved in such a manner that their upper extremities are brought together and fuse with one another on the under surface of the superficial layer; it is this fusion of the pedicels which forms the reticulate thickening of the superficial layer.

By the study of a series of vertical transverse and longitudinal sections of the spiracle, it becomes possible to interpret the relationship of the parts described in the preceding paragraph (Pl. XXII, fig. 3 and Pl. XXIII, figs. 1—10). The continuity of the thin superficial layer is

[1] The terms macula and ostium are both new: the term pore has been very loosely used in general descriptions and appears to refer to the macula. We see, however, that the macula may contain no pore or aperture communicating with the tracheal system.

broken at intervals by the external pores (*e.p.*) and directly beneath the latter are seen the internal pores (*i.p.*) passing through the basal layer. Large pyriform air spaces are formed between the groups of pedicels beneath each of the external pores, and these pyriform spaces (*p.s.*) establish direct communication between the two series of external and internal pores.

The portion of the spiracle beneath the macula shows an entirely different structure. It is occupied by a columnar mass of connective tissue and muscle fibres running upwards from the soft structures underlying the spiracle; this mass of soft tissue, which we have termed the columella, extends up to the under surface of the superficial layer which is considerably thickened at this central portion (Pl. XXIII, figs. 7—10 *col.*). Completely encircling the columella is a large annular air space, the pericolumellar space (*pc.s.*) which communicates directly with the interstices between the pedicels in the intermediate space. The ostium opens into the pericolumellar space on the internal side of the columella (Pl. XXIII, figs. 5—7) and immediately beneath the ostium the cavity of the pericolumellar space is continued downwards as a large chamber of irregular shape which we have termed the atrium (*a.*). The cavity of the atrium is roughly elliptical in cross section, somewhat contracted dorso-ventrally; its direction is at first horizontal or slightly upwards, it then bends downwards and terminates abruptly, the entire length being equal to about half the diameter of the spiracle. Numerous main tracheal trunks (*tr.*) open separately from the basal portion of the atrium, running inwards and downwards for a short distance as a stout bundle of tubes with spirally thickened walls, from which the tracheae radiate to all parts of the body. Running up the columella and inserted along the dorsal wall of the atrium (to the right of the atrium in the figures on Pl. XXIII) is a band of muscle fibres, a small portion of which runs up to the superficial layer and is inserted beneath the centre of the macula. The contraction of this columellar muscle would dilate the cavity of the atrium and possibly at the same time close the ostium, the external margin of which overhangs the slit-like opening. This would cause the inspired air to filter through the external pores of the superficial layer. The expulsion of air from the tracheal system is effected by the contraction of the dorso-ventral body muscles which squeezes the contained air out through the spiracle, a fresh supply being inspired by the elastic rebound of the tracheal tubes, when the action of the body muscles is relaxed.

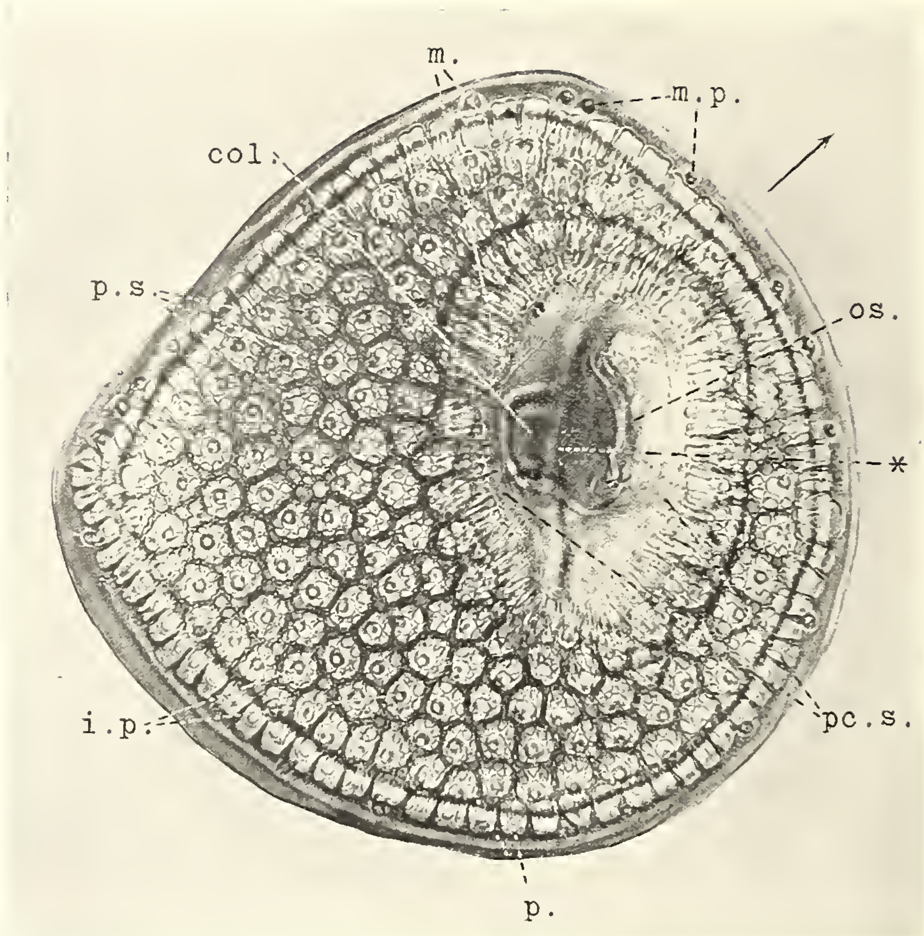

Fig. 1.

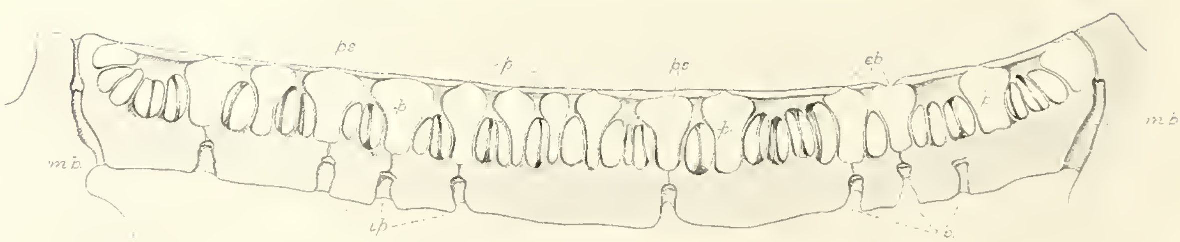

Fig. 3.

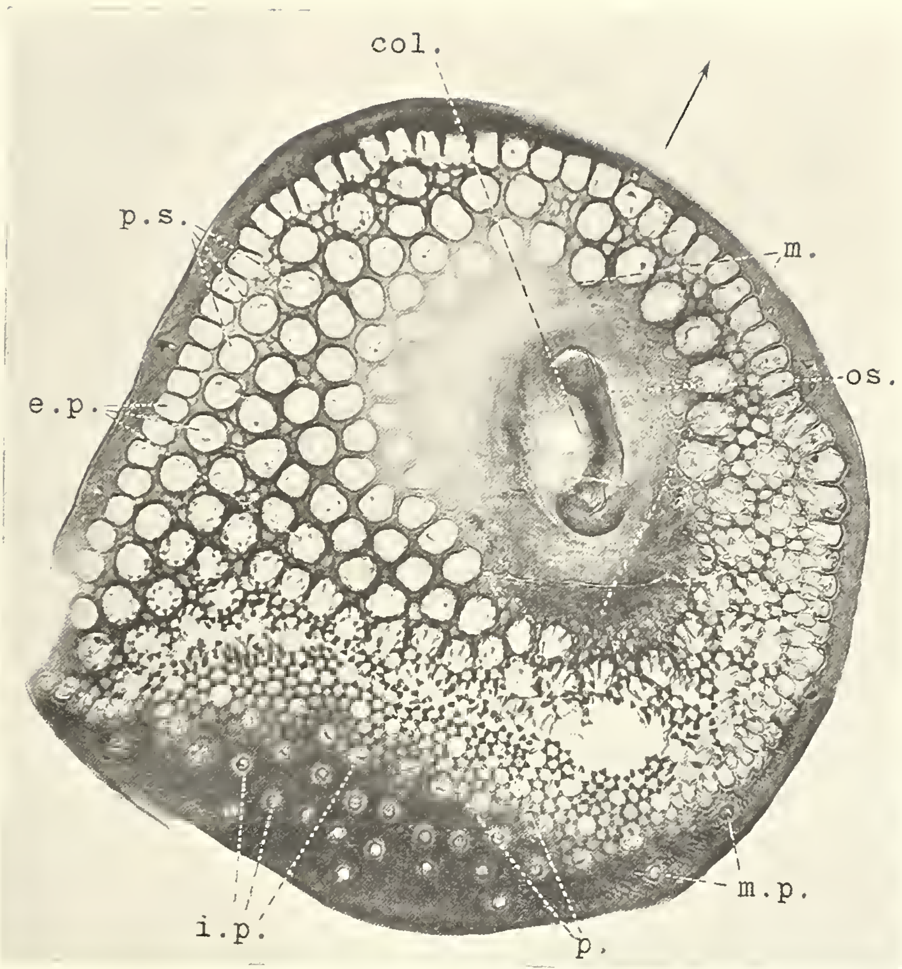

Fig. 2.

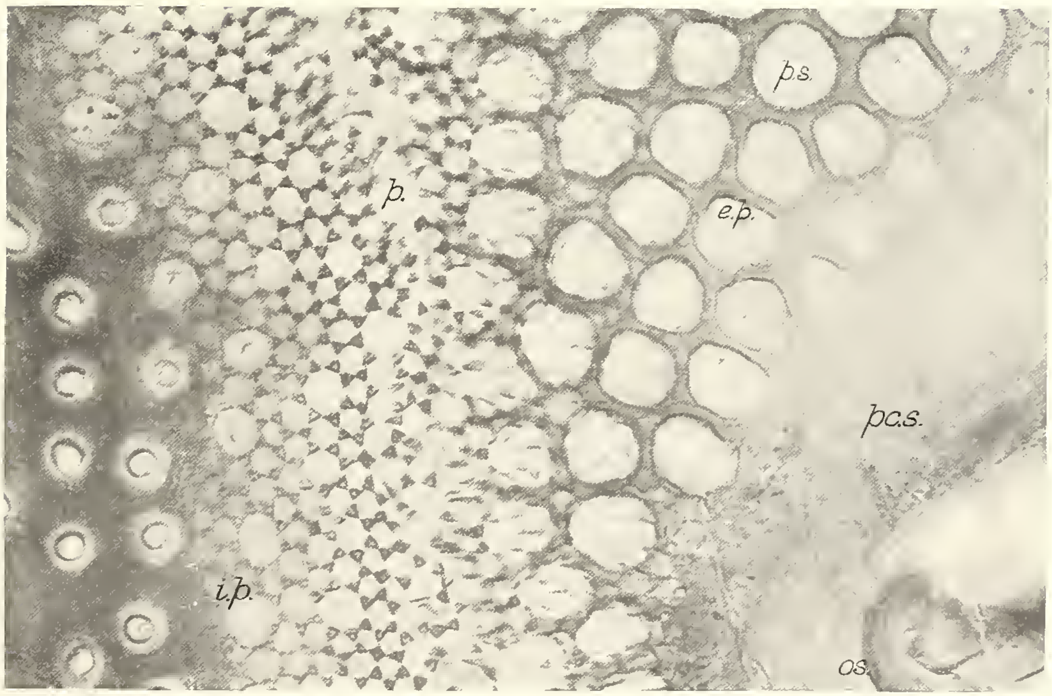

Fig. 4.

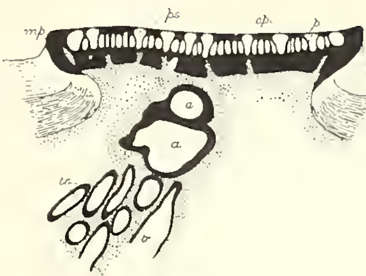

Fig. 1.

Fig. 2.

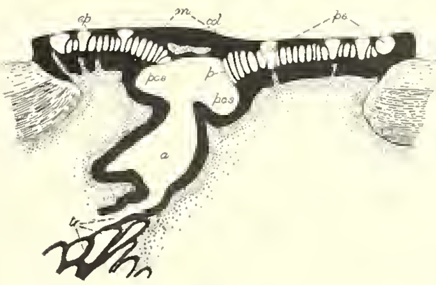

Fig. 3.

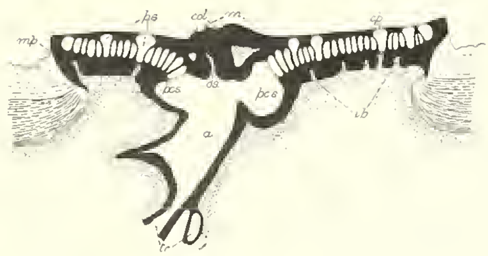

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 7.

Fig. 8.

Fig. 9.

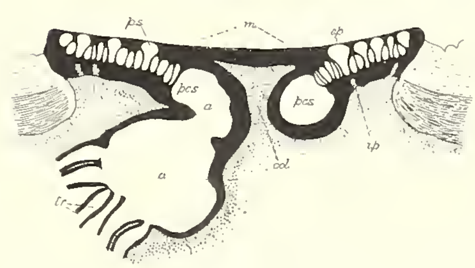

Fig. 10.

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DESCRIPTION OF PLATES XXII—XXIII.

PLATE XXII.

Fig. 1. *Haemaphysalis punctata* ♀. Right spiracle as seen in transmitted light; the arrow indicates the direction of longitudinal axis. Photomicrograph (L. E. R.) × 220.

Fig. 2. *Haemaphysalis punctata* ♀. Horizontal section through right spiracle passing in a slightly oblique plane and cutting the different layers successively; the arrow indicates the direction of the longitudinal axis. Photomicrograph (L. E. R.) × 220.

Fig. 3. *Haemaphysalis punctata* ♀. Transverse section through spiracle (not passing through macula) showing characters of external, internal and marginal pores. (L. E. R. del.) × 300.

Fig. 4. Small portion of Fig. 2, highly magnified, showing regular arrangement of pedicels, etc. Photomicrograph (L. E. R.) × 500.

PLATE XXIII.

Chitinous structures of spiracle—black: general cuticle of body—line-shaded: soft tissues—stippled: air spaces—clear.

Figs. 1—8. *Haemaphysalis punctata* ♀. A series of transverse sections passing through the central portion of the spiracle, showing communications between the various air spaces, the atrium and tracheae, etc. × 100.

Figs. 9—10. *Haemaphysalis punctata* ♀. Longitudinal sections passing through the macula on external side of ostium, Fig. 10 being the more internal of the two. × 100.

INDEX TO LETTERING ON PLATES XXII—XXIII.

a.	atrium.
col.	columella.
e. p.	external pores.
i. p.	internal pores.
m.	macula.
m. p.	marginal pores.
os.	ostium.
p.	pedicels.
p. s.	pyriform spaces.
pc. s.	pericolumellar space.
tr.	tracheae.
* (Pl. XXII, Fig. 1)	indicates external wall of atrium, seen through thickness of spiracular plate.

The arrows on Pl. XXII, Figs. 1 and 2 indicate the direction of the longitudinal axis of the spiracle.

A CONTRIBUTION TO THE LIFE HISTORY OF *ECHINOSTOMUM SECUNDUM*, NICOLL.

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Plate XXIV.

In some notes on the Trematodes of Northumbria published in 1905 a few remarks were made on a larval Trematode inhabiting the liver of the common periwinkle *Littorina littorea.* The liver in two per cent. of the periwinkles from Budle Bay was full of rediæ containing cercariæ more or less developed, the latter agreeing in every way with an encysted *Echinostomum* larva which inhabits mussels, cockles and other bivalve mollusks in the same locality. So close was the resemblance that I had no hesitation in declaring them to be the same worm in different stages, but hoped for an opportunity of demonstrating this by experiment. In October 1908 through the courtesy of Professor Meek I had the opportunity of conducting some feeding experiments in the Dove Marine Laboratory, Cullercoats, which have given satisfactory results, and although it is not possible to state absolutely that the forms are identical yet the evidence is so strong that I think I am justified in regarding the young worm in the periwinkle as an earlier larval form of the encysted worm in the foot of the mussel and cockle.

The youngest stage of this species I have seen is the redia, and I have never noticed miracidia or sporocysts. The liver in the infested periwinkles instead of being of a greenish-brown colour, as it usually is in healthy specimens, is a pinkish orange. On examination this is seen to be due to an enormous quantity of rediæ packed together. So crowded are they that very little liver substance is left and almost the whole of the spire of the shell is occupied by the worms. The full-grown redia is easily seen with the naked eye as a pinkish-yellow sac

about 2·6 mm. long and 0·48 mm. broad. Young rediæ are also present measuring about 0·40 mm. long, or, when extended, about 0·60 mm. These young rediæ are very active and move continually by alternate contraction and extension. They are quite colourless and transparent, have a large muscular oval sucker leading into a strongly developed pharynx and an inconspicuous intestine containing no food material. (See Plate XXIV, fig. 1.) The anterior end of the body is marked with broad wrinkles and this part terminates posteriorly in a "collar." At the tail end are two blunt processes which disappear in the full-grown rediæ. The body is full of masses of cells which are evidently the beginning of the formation of the cercariæ.

The redia at a later period begins to feed, and, as it grows larger, food material is seen in its sac-like intestine as yellow and brown granules (black in the figure). The body becomes pinkish or orange, the striations, collar and posterior appendages disappear; the shape is now simpler and somewhat resembles that of a stocking and the creature is very inert (Plate XXIV, fig. 2). Inside the sac are many cercariæ in various stages of development, and, when full-grown, these break out of the redia at the posterior end.

The cercaria is colourless and transparent and consists of three parts, (1) a heart-shaped head, (2) an elongated body, (3) a very active tail, not quite so long as the body. The length of the worm minus the tail is about 0·70 mm. The tail lashes continually backward and forward and may be occasionally seen detached from the body and moving about in the redia. When the worm loses its tail it moves in a leech-like manner by means of its suckers.

The body is covered, except at the posterior end, with minute spines and the head bears 29 large pointed spines arranged in an incomplete circle. The three last spines on each side are arranged in a peculiar way, one large spine occurring between two which are much smaller than any of the others. These two spines are on a lower level.

The oral sucker is at the extreme anterior end and is much smaller than the ventral which is situated well behind the centre of the body. The mouth leads into a long narrow prepharynx followed by a muscular pharynx and a short œsophagus which divides near the centre of the body into two narrow intestinal lobes; the latter reach to the posterior end of the body. The excretory system consists of an oval posterior vesicle into which open two very much branched lateral ducts which begin one on each side of the oral sucker, curve gently inwards in a small bay and then receive the lateral branchlets. The ducts are

full of highly refractive granules and are the most conspicuous organs in the worm.

A great part of the body is taken up by gland cells. Two straight ducts run forward from these, one on each side of the oral sucker and split anteriorly into smaller ducts which open just in front of the oral sucker. These glands are probably used for the secretion of the cyst since they disappear in the encysted condition (Plate XXIV, fig. 3).

The cercaria is now ready to leave its first host the periwinkle. How it gets out has not been observed. Possibly it enters the alimentary canal and issues from the anus; or it may bore its way through the tissues of its host, or the periwinkle may die and so liberate the cercariæ. The last hypothesis is the least likely as the cercariæ do not all grow up at the same time, sometimes only one or two are ready to leave the periwinkle at one time and sometimes a great many reach this stage at once.

It may be presumed that after leaving the periwinkle the tailed cercaria swims about in the water until it finds its second host in which it settles down and encysts.

The second or intermediate host is the mussel. This is practically proved by the experiments given below. The same encysted cercaria has been found in the foot of *Cardium edule, Mya arenaria* and *Tapes pullastra.* The above description exactly corresponds to the encysted cercaria except that in the latter the tail and glands have disappeared. The cyst is thin-walled and measures 0·2—0·25 mm. across. It is clear and colourless and is easily burst by slight pressure.

The encysted stage of this worm was first briefly and incompletely described by myself (1904) from the cockles at Budle, and later, more fully, by Dr W. Nicoll (1906 *a*) from the cockles and mussels at St Andrews. Afterwards it was found abundantly in both cockles and mussels at Budle. It occurs occasionally in the liver of *Cardium edule* and *Mytilus edulis* as well as in the foot; Nicoll has also observed it round the mantle edge of *Cardium edule.*

Almost every mussel at Budle is infested with this parasite and about ten per cent. of the cockles. The enormous number of the cysts points to a common shore animal being the first host and—the periwinkle abounds in Budle Bay.

The cercaria probably enters the mussel or cockle by the mouth and bores its way into the soft parts of the body. The presence of the cysts in the liver suggests this. Those in the mantle edge may perhaps enter through the epidermis but it seems unlikely that they penetrate

the foot by boring inwards from the outer wall. The epidermis of the foot is very tough and it would probably be difficult for the worm to enter this way.

The cyst is colourless, quite transparent and possesses a thin wall. The cercaria enclosed within the cyst has now lost its tail and head glands but in other respects exactly corresponds with the cercaria from the periwinkle. The spines, suckers and excretory system can be seen through the cyst. The excretory system is very conspicuous.

Nicoll (1906 *a*, 1906 *b*) has shown that the encysted cercaria is in all probability the larval stage of a new species of *Echinostomum* which he names *E. secundum*. This worm occurs in the Oyster Catcher (*Hæmatopus ostralegus*), the Herring Gull (*Larus argentatus*) and the Black-headed Gull (*Larus ridibundus*). It has been found by Nicoll in the intestines of these birds in all stages from small young specimens, agreeing exactly with the encysted cercaria from the mussel, to adults of various lengths, the longest measuring 7·3 mm. Although *E. secundum* resembles *E. leptosomum* Creplin in many ways, it is certainly a distinct species. I have found the latter worm in the intestine of the Dunlin (*Tringa alpina*) and the Turnstone (*Strepsilas interpres*) and, although the specimens were not in a very good state of preservation, it was easy to see that they were certainly distinct from the worm described by Nicoll. Moreover *E. leptosomum* agrees with the encysted larva from *Scrobicularia tenuis* and earlier stages from *Paludestrina stagnalis* while *E. secundum* agrees with the cercaria from the mussel and periwinkle above described. As I have shown (1907) the rediæ of the two species differ in form. The cercariæ agree in almost every point except in size and in the fact that the head spines, although equally numerous in both species (29), in *E. leptosomum* are all almost alike in size. The same difference is seen in the adults and it is constant. *E. secundum* is also much broader compared with its length than *E. leptosomum*, the oral and ventral suckers are larger and the ova are of a much greater size.

We may, I think, look upon the larval forms from the mussel and cockle as the young of *E. secundum* although this is unfortunately difficult to prove. The above-mentioned birds eat the mollusks containing the cysts. The cysts dissolve in the stomach and pass into the intestine where they grow into the adult forms. The Oyster Catcher, which is common at Budle, feeds constantly on the mussels, the stomachs being nearly always full of broken pieces of the shells. The Herring Gull and Black-headed Gull are also common shore feeders at Budle.

Although infection experiments on the birds are almost impossible, I have been able to prove the infection of the mussel from the periwinkle. In experiments of this kind there is always a risk of the second host being already infected, but in this case great care was taken and a control experiment carried on at the same time.

Experimental Infection.

Two tanks A and B were used for the experiment. Sea water from Cullercoats Bay ran into these continually. The water was evidently free from the parasite as this worm has never been found at Cullercoats and almost certainly does not occur there.

The mussels (350) were procured from Blyth Harbour. They were specially gathered from wood-work and piers at least fifteen feet from the ground and from a locality where the mussels have never been known to harbour the parasite. Of these mussels, 50 were opened and examined microscopically and were found to be free from the parasite. The remaining 300 were divided into two portions, one of which was put into Tank A and the other into Tank B. Into Tank B were also put 300 periwinkles from Budle Bay, and one periwinkle which had been cracked open to show that it contained the parasite. These experiments were started on October 19th, 1908. Mud and a little seaweed from Cullercoats Bay were from time to time put into both tanks.

At intervals of about a week mussels from each of the tanks were forwarded to me at Leeds for examination, with the result that out of 30 mussels from each tank, 24 from Tank B contained the encysted worm but none of those from Tank A were infected. The number of cysts in each infected specimen varied from one to five and all were in the foot with one exception when the parasite occurred in the liver. As the mussels of the control experiment in Tank A were in no case infected it seems clear that the source from which the parasites came was the periwinkles, and that the *Echinostomum* encysted in the mussel's foot is a later larval stage of the cercaria contained in the periwinkle. The life history of *Echinostomum secundum* may therefore be summed up as follows:—

First Host	Second or Intermediate Hosts	Final Hosts
Littorina littorea	*Mytilus edulis* *Cardium edule* *Mya arenaria* *Tapes pullastra* *Mactra stultorum*	*Hæmatopus ostralegus.* *Larus ridibundus.* *L. argentatus.*

A detailed list of the experimental mussels showing the number opened and the parasites contained in them is here given.

Table showing the number of mussels examined and the Trematodes they contained.

Date	Tank B		Tank A	
	No. of mussels examined	Trematodes found	No. of mussels examined	Trematodes found
30 Oct.	6	*a.* 0 *b.* 0 *c.* 0 *d.* 0 *e.* 1 in liver *f.* 1 in foot	6	0
6 Nov.	5	*a.* 0 *b.* 1 in foot *c.* 2 ,, *d.* 2 ,, *e.* 3 ,,	5	0
18 Nov.	6	*a.* 2 in foot *b.* 2 ,, *c.* 3 ,, *d.* 3 ,, *e.* 4 ,, *f.* 5 ,,	6	0
26 Nov.	6	*a.* 3 in foot *b.* 1 ,, *c.* 2 ,, *d.* 0 *e.* 1 in foot *f.* 1 ,,	6	0
8 Dec.	6	*a.* 5 in foot *b.* 5 ,, *c.* 5 ,, *d.* 3 ,, *e.* 3 ,, *f.* 1 ,,	6	0

My best thanks are due to Miss A. M. Carr who has kindly looked after the experiments and forwarded specimens to me from time to time. I am likewise indebted to Captain G. J. Robinson, Harbour Master at Blyth, who kindly procured me the mussels.

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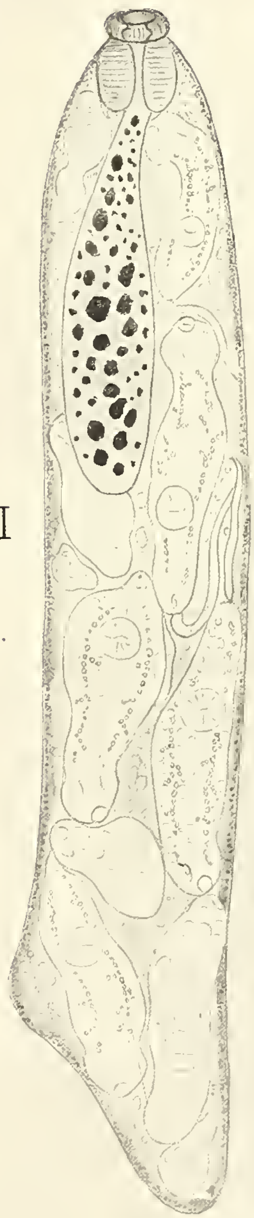
EXPLANATION OF PLATE XXIV.

Fig. 1. Young redia of *Echinostomum secundum*.

Fig. 2. Full grown redia containing Cercariæ.

Fig. 3. Front of head of Cercaria. (S = ducts of glands, E = excretory duct. The head spines are omitted for clearness.)

II



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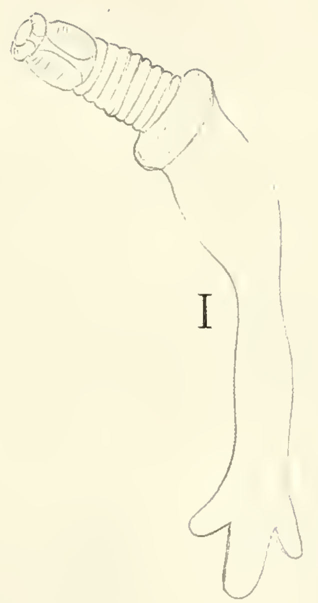

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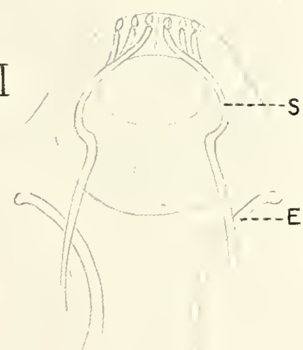

FILARIA VOLVULUS LEUCKART, ITS DISTRIBUTION, STRUCTURE AND PATHOLOGICAL EFFECTS.

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(One Figure.)

Although *Filaria volvulus* was first described by Leuckart in 1893, very little was known or taught of this particular nematode when, ten years later, I became a government official in Northern Nigeria. While other members of the Filaridae have received a good deal of attention during the present decade, *Filaria volvulus* seems to have been comparatively ignored. I am inclined to think, however, that this Filaria is far more common in certain parts of Africa than is generally supposed; and, by publishing the cases that have come under my notice, I hope that something may be added to our knowledge of the obscure disease it causes and that other workers will become interested in the subject. Up to the present time I have seen five cases, and all of these came under observation at Lokoja—a large government station in Northern Nigeria, situated at the confluence of the Niger and Benue rivers. To my knowledge this is the first time that cases of *Filaria volvulus* have been reported from Northern Nigeria.

Literature. To Leuckart belongs the honour of first describing and naming this filaria. His material was supplied him, by a German Missionary, from the Gold Coast. Six years later Labadie-Lagrave and Deguy (1899) described a young female worm found in the case of a French soldier who had been quartered in Tonquin. Subsequently Brumpt (1904) added to our knowledge of this worm: he also considered that *F. volvulus* is a common parasite in certain inland districts of

Africa. Previously to this, Prout (1901) had given a detailed description of two worms found in a native of Sierra Leone. Finally Fülleborn (1908) wrote a critical article, illustrating the text with some interesting photomicrographs and diagrams. Besides contrasting his observations with those of Prout and others, this writer is inclined to think that the Cameroons has supplied him with a new species of filaria.

Occurrence. It will be noticed at once that Africa has supplied all the cases of *F. volvulus* hitherto reported. Moreover, with the exception of four cases recorded by Cooke in Uganda, the distribution appears to be limited to Western Tropical Africa. My own experience, too, corresponds with that of other observers, who lay stress on the fact that the subjects of *F. volvulus* are usually inhabitants of riverine districts. Fülleborn (1908), on the authority of Kulz, states that in the valley or district of the Wuri about 10 % of the men are infested. This would seem to show that *F. volvulus* is a common parasite in certain parts of the Cameroons. The neighbourhood of the Welle river in the Congo State also appears to be associated with the parasite, though according to Manson (1908) *F. volvulus* is not found on the Congo river: further investigations will probably modify this statement. Practically all the West African Colonies have contributed cases of *F. volvulus*, and we find the disease to which it gives rise reported as existing in the Congo, the Cameroons, Dahomey, Gold Coast, Sierra Leone and lastly, Nigeria. Dr Leiper informs me that the parasite has also been found in Uganda, so that, for the present, *F. volvulus* appears to be limited in a peculiar manner to the equatorial zone of the 'Dark Continent.' This may be due to lack of observation and insufficient data, or to a curious distribution of the intermediate host. Possibly both causes are at work. Personally, I venture to think, that, if every little subcutaneous tumour occurring in the negroid inhabitants of tropical Africa were examined *ex corpore*, it would be found that this form of filariasis was not at all uncommon. It is hardly necessary to say that in races so addicted to glandular enlargements as the African races are, mere casual observation is useless; consequently the suspected tumours must be excised for purposes of examination and diagnosis.

The subjoined table shows the chief clinical features of the five cases that form the subject of this paper.

	Case I	Case II	Case III	Case IV	Case V
Age ...	23	36	45	12	10
Sex ...	M	M	M	M	F
Occupation	Prisoner formerly a farmer	Prisoner formerly a farmer	Prisoner formerly a farmer	School boy	Freed slave ward
Race	Gadi (Pagan)	Igbirra (Pagan)	Igbirra (Pagan)	Onitsha (Pagan)	Fulani (Mahommedan)
District	German Cameroons	Kabba N. Nigeria	Kabba N. Nigeria	Onitsha S. Nigeria	Tola Benue River
Characteristics of Tumour	Multiple chest wall and scalp	Multiple chest wall	Multiple chest wall	Single Chest wall	Multiple flank adherent to iliac crest, size of hen's egg
Blood examination	*Filaria perstans* found in blood	(Day) Nil	(Day) Nil	(Day & night) Nil	(Day & night) Nil
Remarks	No inconvenience from tumours, similar cases said to be common in his village	No inconvenience or symptoms	No inconvenience or symptoms	No inconvenience or symptoms. A brother said to have similar tumours	No inconvenience or symptoms

Pathological Effects of Filaria volvulus.

One of the most interesting features in the natural history of *F. volvulus* is the formation of subcutaneous fibroid tumours which vary in size from a split pea to a hen's egg. Clinically they are somewhat suggestive of dermoid tumours at first sight, being freely moveable over the subjacent tissues, while they are more or less firmly attached to the overlying skin. In older tumours, however, there may be very little mobility, and in one of my cases the growth was tightly adherent to the iliac crest. Apparently these tumours give rise to no symptoms, local or general; also, they must be very chronic in character judging from what I have gathered from the subjects of the disease. In my experience suppuration never occurs and Fülleborn has come to the same conclusion. As regards the position of the tumours Fülleborn (1908), quoting Zupitza, states that no part of the body is exempt, and they have been noted in such regions as the scalp, the chest wall, and the gluteal parts.

It is interesting to note that the observations of Kulz (cited by Fülleborn, 1908) are in accordance with mine in determining the

neighbourhood of the floating ribs as the favourite site for these tumours. Enucleation is usually not difficult though care should be taken to remove the tumours intact. As a rule, the excised growth is found to consist of two or three tumours of varying sizes all closely invested with a strong dense fibrous capsule. On section the composition of these tumours soon becomes apparent, and the peculiar testicular feeling experienced on palpation is explained. An opaque grumous fluid exudes from the tumour which is now seen to be composed almost entirely of an inextricable meshwork of filarial worms. These would appear to be most closely interwoven in what might be called the cortex of the tumour, while usually there is a central cavity which is bridged in every direction by the more or less disengaged portions of male and female worms.

Attempts to tease out portions of the tumour are attended with great difficulty owing to the manner in which these fragile little nematodes lie imbedded in the fibrous stroma. This fibrous tissue is probably formed as a result of reaction against the presence of the worms; and the fact that the adult *F. volvulus* has a rough and transversely corded cuticle makes it easy to account for the irritation that is set up. Or, it may be that the fibrosis is the result of some special secretion or excretion on the part of the worm. The fluid found in these tumours seems to vary somewhat according to the age of the tumour. In young tumours the fluid is scanty in quantity, and of a semi-opaque milky appearance. In older and larger tumours, on the other hand, it becomes thicker and more opaque, and takes on a dirty yellowish tinge; it may even assume a reddish colour as Zupitza has pointed out (cited by Fülleborn, 1908). Microscopical examination reveals the fact that the fluid of these tumours contains ova and embryos in large quantities together with fatty particles and cellular débris. As regards the adult inhabitants there seems to be a preponderance of females over males, but anything like an accurate count is well nigh impossible. In all probability a tumour 1 inch long would contain at least four females and three males, and here I think an observation in Prout's paper (1901) calls for some criticism. This observer states that he only found one female in a tumour 1 inch long and, on piecing the bits together, it measured 16 inches. It seems very doubtful whether a worm so narrow and slender would attain such a length, and it appears probable that the pieces belonged to several females. The small size of the male, too, rather supports this view as it is usually about half the length of the female.

The Anatomy of Filaria volvulus.

The mature worms are delicate cylindrical organisms of a greyish white colour, and are always found in a coiled up condition. They possess a thick and rigid cuticle which is annulated for the greater part of the body and is only seen broken in a transverse direction. The male worm measures from 20—32 mm. in length, and ·2 mm. in diameter at its widest part. The female is both longer and thicker than the male, about twice as long, and measures ·3 mm. in its thickest part.

The alimentary system is represented anteriorly by a mouth that opens at the bottom of a very small cup-like depression having a cuticular lining continuous with the cuticle of the body. There are no circumoral papillae. The oesophagus is a stout tube possessing a cuticular lining and measuring ·8 mm. in length. The rest of the alimentary canal is a thin walled straight and narrow tube filled with opaque matter, which seems to show that the animal feeds on organised tissues, and does not wholly depend on lymph.

The *male worm* is hair like in form and maintains the same diameter throughout the greater part of its length. A very characteristic feature of the male is the single spiral twist at the end of the tail which is seen in all specimens. With care the male worm can be disentangled completely from the tumour and we found that the shortest worm measured 20 mm. while the longest was 32 mm. The cuticle is very finely ringed and resistant. The tail measures 0·07 mm. and ends in a bulbous blunt portion.

Fülleborn (1908) states that the tip of the tail is inverted to make a gutter like depression, but it is probable that this sulcus is apparent only, and is produced as a result of the arrangement of the caudal papillae. At 0·07 mm. from the head, the body of the male worm narrows to form a slightly narrower neck which is 0·05 mm. in diameter; thereafter the diameter increases uniformly until about the middle of the body the greatest width of 2 mm. is reached. Two groups of paired papillae occur at the posterior end of the worm:—(*a*) those near the anogenital pore or cloaca, and (*b*) those near the tip of the tail.

Concerning the number and arrangement of these papillae there is much diversity of opinion among the various authors, but our conclusions are based upon an examination of several specimens and are as follows.

In group *A* there are two pairs of pre-anal, and two pairs of post-anal papillae, all lying close together and almost touching one another. Occasionally the third papilla (i.e. the one immediately posterior to the cloaca) is situated more internally than the other three pairs, thus giving the deceptive appearance of three pairs only. This apparently has misled Fülleborn since he concludes that there are only three pairs. Group *B* contains two pairs of papillae situated at the tip of the tail; one pair being subterminal and ventral, and the other pair terminal and lateral. Midway, however, between the tip of the tail and the cloaca we have noted another large papilla, situated somewhat on the left side of the middle line, but having no corresponding papilla on the right side. The characters of this papilla, including the presence in it of a nerve fibril, leave no room for doubt that it is a true papilla, and not an artifact. The spicules are two in number and unequal in shape and size. They have been well delineated by Fülleborn. The larger spicule measures ·23 mm. in length and ends in a sharp fluted point: the smaller measures ·08 mm. in length and ends in a club shaped knob.

Filaria volvulus ♂.

Table of comparative measurements.

Author	Length of worm	Length of large spicule	Length of small spicule	Tip of tail to cloaca	Greatest diameter	Diameter of head
Prout	30·25 mm.—30·35 mm.	—	0·082 mm.	—	—	—
Braun	30—35 mm.	—	—	—	0·14 mm.	0·04 mm.
Fülleborn	—	0·166 mm.	0·08 mm.	0·07 mm.	—	0·048 mm.
Parsons	20—32 mm.	0·23 mm.	0·08 mm.	0·07 mm.	2 mm.	0·04 mm.

Female. The extraction of a whole female worm is a matter of the greatest difficulty, and we were unable to obtain a complete specimen. This difficulty has been also experienced by other workers. Though they give certain lengths, there is room for doubt as to the correctness of their figures. Thus Prout's measurement of 16 inches has already been noticed (p. 362); Leuckart's specimen measured 60—70 cm., while Védy (1906) gives 18 cm. as the length of his specimen. The longest portion of female worm that we were able to obtain measured four inches.

The greater part of the female worm has a uniform width of 0·3 mm. but the anterior end becomes narrow and whip like, differing very little

in size and measurement from the same part in the male. We were not successful in our attempts to obtain the tail extremity of a female. In the middle of the worm the cuticular markings are more sharply defined and wider apart than in the male. Fülleborn rather happily compares the appearance of these cuticular striae to that of the wooden bands or hoops round barrels. He also lays great stress on the fact that Prout's female specimen did not apparently possess the same pronounced cuticular thickenings that his own specimen showed, and is rather inclined to believe that his own specimens constitute a new species. This seems improbable in view of the fact that his description of the male worm differs but very little from Prout's description of the male. Towards the extremities these cuticular striae in the female become finer and more closely set, and near the vulva they almost cease to exist. At 0·65 mm. from the head the genital pore opens without any marked protuberance of its lips. The vagina passes directly backwards as a thin walled canal, but shows a slight retort shaped dilatation just before its narrow aperture. The uterine tubules have thin cuticular walls with curious spiral markings not unlike the spiral vessels of vegetable morphology. Embryos were found at various stages of development in these tubules:—at one point coiled within the egg-shell; elsewhere partially coiled and distending the egg-shell almost to rupture; while in terminal portions of the tubule they were quite stretched out. These latter, like the embryos found in the fluid of the tumours, possess no sheath, a point on which all observers

Filaria volvulus ♀.

Table of comparative measurements.

Author	*Length of worm	Greatest diameter	Diameter of head	Distance of genital pore from head
Leuckart	60—70 cm.	—	—	—
Labadie-Lagrave and Deguy	25 cm.	0·15 mm. (immature worm)	—	—
Védy	18 cm.	—	—	—
Prout	16 in. or 40 cm.	·36 mm.	0·04 mm.	—
Fülleborn	—	·33 mm.	At ·005 from mouth = ·065 mm.	·55 mm.
Parsons	4 in. or 10 cm.	0·3 mm.	—	0·65 mm.
Brumpt	—	—	—	0·760 mm.
Braun	60—70 cm.	0·36 mm.	0·04 mm.	—

* The measurements here recorded relate to the length of portions only of the female worm: moreover many of the figures must be taken with considerable reserve.

seem to agree. The embryos of *F. volvulus*, however, have a much thicker cuticular integument than is seen in other microfilariae of man, and the cuticle is transversely striated.

The *Embryos* measure 24 mm. in length. In stained specimens we were able to make out the central core of nuclei as described in the microfilariae. These granules, however, appeared to be more numerous and smaller than usual and we were not able to determine definitely the 'breaks' in the core corresponding to the rudiment of the nerve ring, to the excretory pore, and genital stolon.

Development. All observers agree that these worms live in local dilatations of lymphatics, and that most probably the filarial embryos pass from these into the blood stream and are transmitted by biting insects. No observer has however detected the embryos in the blood. My own experience confirms these observations. Although it was evident from an examination of the contents of the tumours that millions of embryos were being discharged at the time of removal of the tumour I failed to find in repeated examinations any sign of these in the blood.

The distribution of the disease, as at present known, suggests the existence of a riverine intermediary, but of the further development of the parasite nothing is at present known, and this remains a problem for the future.

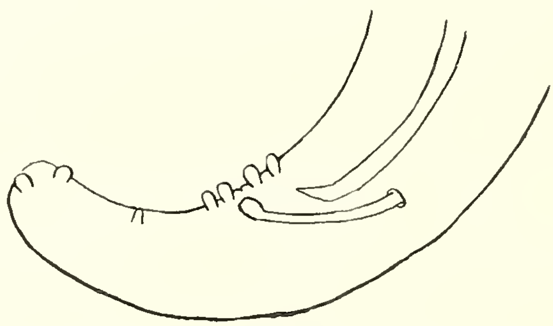

Fig. 1. *Filaria volvulus* Leuckart ♂.

Posterior end of a male specimen showing disposition of the papillae as seen from the left side. The solitary papillae lying midway between the peri-anal and the caudal groups is unpaired. The larger spicule is not shown in its entirety.

Symptoms and Treatment. As already stated, the presence of *F. volvulus* in the human subject appears to give rise to no symptoms or even inconvenience. The subjects of these tumours regard them as harmless possessions, and usually dismiss the matter with the remark that the tumour has existed for a very long time, and no longer con-

cerns them. To my knowledge pyrexia is unknown in connection with this form of filariasis. The only symptoms likely to occur are those caused by pressure. In one of my cases, I was asked to remove a tumour because it was unsightly and interfered with the proper disposition of the child's clothes. Treatment is simple and, if the 'disease' is merely local, effective. The tumours in themselves are easily enucleated with the aid of a little local anaesthesia: all the growth should be removed cleanly and intact.

Conclusions.

1. Time will probably show that *F. volvulus* is more common than has hitherto been supposed.

2. Analogy would lead us to suspect that *F. volvulus* is transmitted by some blood-sucking insect.

3. The geographical distribution of *F. volvulus*, as at present known, seems to correspond more or less with regions in Tropical Africa that are associated with such insects as have been proved to act as carrying agents in other parasitical affections.

4. While the adult male worm has been studied more or less completely, this does not hold for the female worm. The difficulty lies in the extraction of the worm from the tumour: the tail portion is nearly always left imbedded in the fibrous stroma and defies detection. What seems to be needed is some macerating medium in which the tumour would disintegrate without the worms becoming destroyed.

As regards preservatives a weak solution of Formalin proved very satisfactory, in the author's case, for preserving the tumours during transit.

5. Although the embryos of *F. volvulus* have not yet been found in the peripheral blood, it seems highly probable that some part of their existence must be spent in the general circulation.

Finally, I should like to acknowledge the kindness of the authorities at the London School of Tropical Medicine who allowed me to work in their excellent research laboratories. To Dr Leiper in particular I am much indebted, not only for the valuable help he so generously extended me, but also for his courtesy and friendly criticism.

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THE SCHIZOGREGARINES:
A REVIEW AND A NEW CLASSIFICATION.

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(Nine Figures and one Diagram.)

CONTENTS.

I. Introduction.

At the present time, when so much interest is being displayed with regard to the study of the Protozoa, there is danger of too much attention being centred on those Protozoal organisms which give rise to disease in man and in the higher animals, to the neglect of many most interesting parasitic forms which do not necessarily give rise to disease,

and which consequently are not counted among the pathogenic Protozoa. The study of the life-histories of these relatively harmless organisms, in addition to being most interesting, may, however, throw light upon the biology of pathogenic forms. Among the former may be reckoned the Schizogregarines, a group of organisms of diverse external form, which are united by one special feature connected with their reproduction, namely, the intercalation in their life-cycle of an asexual method of multiplication. This feature serves to distinguish them from the common Gregarines, in which sexual reproduction alone obtains. The Schizogregarines have recently been the subject of several important papers by a small number of protozoologists.

The object of the present review is to draw wider attention to this very interesting group, and to put forward suggestions—more especially in relation to their classification. Having personally worked at the group, on the *Selenidiidae,* and being acquainted at first hand with *Siedleckia, Aggregata* and *Merogregarina,* I feel that the time has arrived when a review of the group will prove useful, especially since the literature relating thereto is both widely scattered and difficult to interpret.

A Glossary of Terms (see Appendix, p. 411) has been added for the general reader, at the request of the Editors.

II. Historical Survey.

The term "Schizogregarine" dates from the year 1900, when it was introduced by Léger. Parasites belonging to the sub-order *Schizogregarinae* were known, however, before this date, for Aimé Schneider described the first of these forms, *Ophryocystis buetschlii* in a preliminary note (1883), and subsequently (1884) gave fuller details regarding it. The body of this parasite, which occurs in the Malpighian tubules of a beetle, is irregular in shape and possesses pseudopodium-like processes (Fig. 4). Schneider was doubtful as to the position which should be assigned to these parasites, and, on account of the somewhat peculiar feature just mentioned, which he considered diagnostic, he called them the *Amoebosporidia.* As such they are described in the works of Wasielewski (1896), and Labbé (1899) on the Sporozoa, being placed in the appendices[1] as a separate and problematic order

[1] The supposed parasite of cancer was once referred to the problematic group *Amoebosporidia* (see Minchin, 1903, p. 191) and that of variola and vaccinia was in 1895 placed in a genus *Amoebosporidium* by L. Pfeiffer.

of Sporozoa. A correct appreciation of the value of the amoeboid character of the organism in the two species of *Ophryocystis* (*O. buetschlii*, *O. francisci*) then known was first attained by Léger in 1900, when he described an allied parasite, which he called *Schizocystis gregarinoides*, from the gut of the larva of the fly, *Ceratopogon*. *Schizocystis* has a fixed and definite contour, and Léger showed that the so-called pseudopodia of *Ophryocystis* were merely stiff, root-like processes, for fixation of the extracellular parasite to the cells of the gut wall (Fig. 4, A, B).

In 1898 Caullery and Mesnil described a parasite, *Gonospora longissima*, in which an intracellular stage of asexual multiplication or schizogony occurred. In 1907 Brasil published his account of *Selenidium caulleryi* from the digestive tract of the Polychaete, *Protula tubularia*, and definitely placed the genus *Selenidium* (of the new family *Selenidiidae*) in the *Schizogregarinae*, pointing out that those forms of the parasite which divided asexually were intracellular in habitat, in contradistinction to the extracellular character of those of *Ophryocystis* and *Schizocystis*.

Léger (1907) at this time published a paper on the genus *Ophryocystis*. Recently there has appeared the paper by Léger and Duboscq (July, 1908) on *Aggregata* and "*Eucoccidium*," wherein the former (*Aggregata*, till recently considered as a gymnosporous Gregarine of crabs) is shown to be the schizogonic or asexual multiplicative cycle of a Schizogregarine, which passes through its spore-producing cycle in the gut-epithelium of cuttlefish (as the so-called "*Eucoccidium*" or *Benedenia*). Still more recently a detailed study of a new Schizogregarine has been completed by my friend and fellow worker, Miss Annie Porter (August, 1908)[1]. The parasite described by Miss Porter has been named *Merogregarina amaroucii*, and it occurs in the gut of a composite Ascidian from Australia. Other Schizogregarines belonging to the *Selenidiidae* have been described by Brasil and by the present author (1907) in various Annelids.

III. OCCURRENCE OF THE VARIOUS GENERA.

The Schizogregarines almost invariably occur in the lumen of the gut of their hosts or in a channel leading therefrom, such as the Malpighian tubules in Insects. So far as present knowledge goes they

[1] The paper is now in the course of publication; a preliminary communication was published in Oct. 1908.

are more especially prevalent in the two great phyla, the *Arthropoda* and the *Annelida*. They are also known in *Mollusca* and *Ascidiacea*.

The genera *Ophryocystis* and *Schizocystis* occur among the *Insecta*. Various species of *Ophryocystis* are found in the Malpighian tubules of beetles belonging to the genera *Blaps, Akis*, and *Olocrates*, whilst *Schizocystis*, of which only one species, *S. gregarinoides*, is known, is found in the gut of the larva of a species of *Ceratopogon*, a dipterous Insect occurring in the Alpine Lake Luitel.

Various species of *Selenidium* have been recorded from Polychaetes: *Protula, Spio, Scololepis, Serpula, Dodecaceria*, etc. *Selenidiidae* were described from the Gephyrean *Phascolosoma* in 1907, and last spring (1908), while working at Banyuls, I observed two forms of Selenidiid parasites in the gut of the Terebellid, *Polycirrus aurantiacus*. Of the species of *Selenidium* from the above-mentioned Polychaetes many require re-investigation, for some of them were described many years ago by Ray Lankester (1863), Giard (1884) and others, the description often applying only to what is now known as the trophozoite phase, for *Selenidium* is characterised by the presence of well-marked longitudinal myonemes.

Merogregarina amaroucii Porter (1908) in the alimentary tract of the composite Ascidian, *Amaroucium* sp. (from Port Jackson, New South Wales), is the only example known up to the present of a Schizogregarine from a Protochordate. No true Schizogregarine has yet been recorded from the Chordates.

A number of species of *Aggregata* have been described recently by Léger and Duboscq as "coelomic" Gregarines which occur in certain crabs of the genera *Portunus, Eupagurus, Inachus*, etc. The sporogonic phases of the life-histories of these parasites have now been shown by Moroff, and Léger and Duboscq to occur in the Cephalopod molluscs, *Sepia* and *Octopus*. Moroff (1908) has described a number of new species from the octopus.

The stages of the organism formerly known as the gymnosporous Gregarine, *Aggregata* (Frenzel and Labbé), are the schizogonic stages of a parasite, whose sporogonic stages were formerly known as *Eucoccidium* or *Benedenia* or *Légerina* in Cephalopods. As the distribution of the Schizogregarines appears to be somewhat scattered, I have, for the sake of clearness and brevity, placed the main facts regarding them in tabular form (see p. 397 *et seq.*).

The effect of Schizogregarines on their hosts is chiefly to cause the

destruction of epithelial cells of the digestive tract; it is probably not of a serious nature.

IV. General Morphology.

The youngest stage in the life-cycle of the Schizogregarine is that of the *sporozoite* (Figs. 1, 3, I) a minute, protoplasmic body with a distinct nucleus. It is usually somewhat sickleshaped, and measures 5μ to 12μ in length. As it does not differ markedly from the sporozoite of other Gregarines (collectively known nowadays as Eugregarines) its detailed structure need not be further considered. The sporozoite attaches itself by its rostrum or pointed end to an epithelial cell of the gut or lining of the Malpighian tubule, and grows. During this period of growth the parasite absorbs nutriment from its host, and it is known as a *trophozoite* (Minchin, 1903, p. 156). The shape of the trophozoite varies, and as in *Ophryocystis*, and to a less extent in *Schizocystis*, it is quite different from that of the other Schizogregarines and nearly all Eugregarines, it will be well to consider separately the trophozoite of each genus.

Ophryocystis.

The sporozoite in *Ophryocystis* grows, becomes pyriform and applies itself to the surface of the epithelium (Fig. 1, II). It then sends out stiff processes which serve to attach the parasite to the epithelium of the Malpighian tubules of the Coleopteran host. The trophozoite becomes somewhat conical in shape, and, while growing, its primitive single nucleus divides, so that it becomes multinucleate (Fig. 1, III). It is now known as a *schizont*, for cytoplasm gathers around each daughter nucleus and then the whole schizont (Fig. 1, IV) divides into small, uninucleate, somewhat pyriform masses (Fig. 1, V) termed *merozoites* ("schizozoites" of Léger). These migrate into the lumen of the tubule, and later attach themselves between new host cells by means of their processes, and so start a new infection in the same host. The multinucleate schizonts, which divide by multiple fission, are termed by Léger "mycetoid schizonts" (Fig. 1, III), to distinguish them from another form of schizont to be noted presently. The "mycetoid schizonts" somewhat recall the trophozoites of *Myxosporidia* in general appearance, but the resemblance is merely superficial. There is probably no close genetic relationship between these "mycetoid schizonts" and the *Myxosporidia.*

Schizogony continues only for a limited period, the merozoites growing into new schizonts. Towards the end of this period, when the host begins to react upon the parasites, the schizonts grow but exhibit only a few nuclei, some two to four (Fig. 1, VI, VII). These

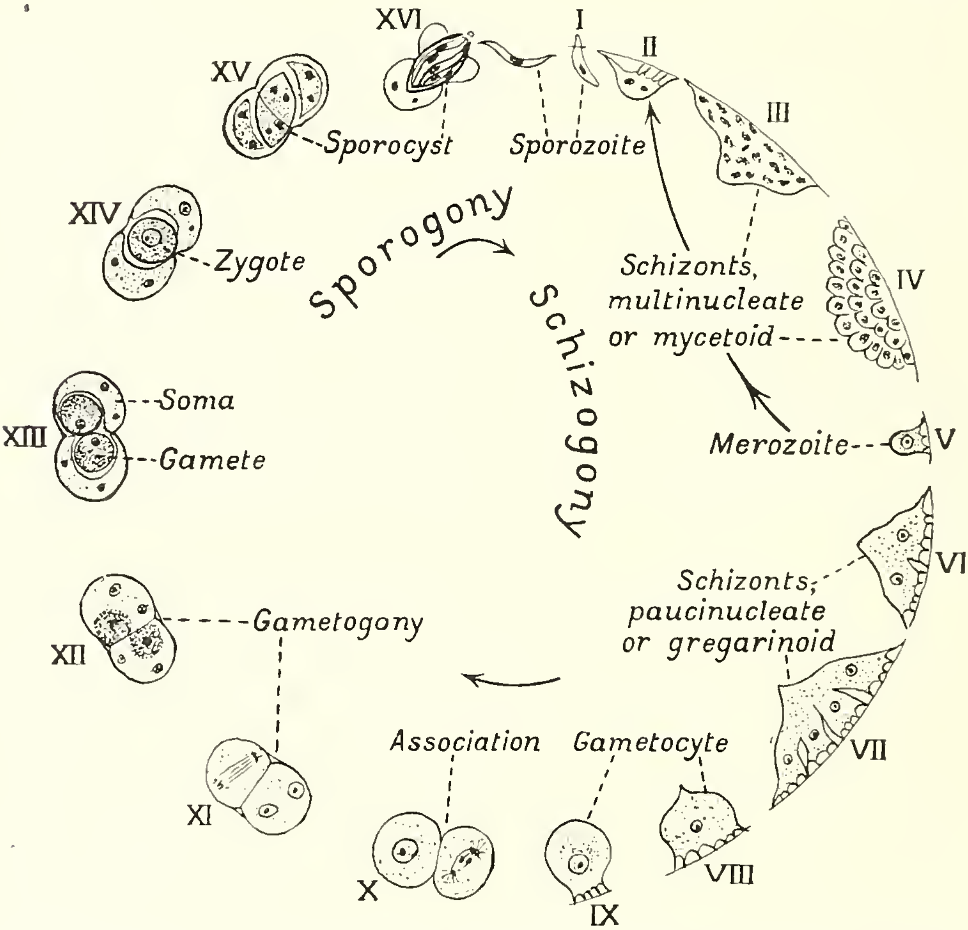


Fig. 1. Diagram of the life-cycle of *Ophryocystis* (based on that of *O. hessei*), after Léger (1907). Terminology altered. II—V, schizogony. VI—XIII, gametogony. XIV—XVI, sporogony.

"paucinucleate" forms, the "gregarinoid schizonts" of Léger, finally break up into *gametocytes* or sexual individuals ("gamontes" of Léger). Each gametocyte (Fig. 1, VIII) possesses only a single nucleus, and its growth goes on without further division until the parasite assumes a globular form (Fig. 1, IX) and becomes detached from the epithelium. It will be noticed that the growth of all the schizonts is *extracellular* and not within the living epithelium of the Malpighian tubule. This

does not occur in the *Selenidiidae*, and it is a point of interest and importance.

The gametocytes of *Ophryocystis* associate in pairs (Fig. 1, X). Nuclear division takes place (Fig. 1, XI), then nuclear reduction, and the two *gametes* (Fig. 1, XII, XIII) so formed, copulate (Fig. 1, XIV) and produce a *zygote*, which becomes a single octozoic spore (Fig. 1, XV, XVI). The details and significance of these stages will be considered later (see pp. 381, 382 and Fig. 4, D).

Schizocystis.

The trophozoite of *Schizocystis gregarinoides* Léger (1900) also needs special mention. It is stated to be of large size, some 150μ long, elongate, cylindrical in shape, with a hyaline, anterior portion, the whole being non-septate or monocystid. It is multinucleate, and the ectoplasm possesses a longitudinally striated cuticle, with a yellowish endoplasm containing refringent granules and rod-like bodies. The trophozoites are attached by anterior sucker-like ends to depressions in the wall of the intestine of the host. The number of nuclei in the trophozoite, which is really a schizont, may be as many as sixty, and the number increases with the size of the schizont, which starts by being a uninucleate, hyaline sporozoite. The schizont divides into a number of merozoites, some of which may be from 20μ to 25μ long, each with a single nucleus. The merozoites form uninucleate trophozoites of the second generation which are the gametocytes. Association and then encystment take place, and conjugation occurs between the gametes. In this way many octozoic spores are produced, after the manner common to the Gregarines.

Selenidium.

As a type of the remaining forms of Schizogregarines with *intracellular* schizogony, that of *Selenidium* may be considered. Species of *Selenidium* inhabit the digestive tract of various Annelids. One of the best known species is *Selenidium caulleryi*, the trophozoite and schizogonic stages of which have been fully described by Brasil (1907). This parasite occupies the digestive tract of *Protula tubularia*, and the infection is a heavy one. The trophozoite of *S. echinatum* (Fig. 2) from *Dodecaceria concharum* has also been well described by Caullery and Mesnil (1899).

The trophozoite of *Selenidium* is an elongate, vermiform, uninucleate organism (Fig. 2, A), roughly circular in transverse section, about 75μ long and 25μ broad in *S. caulleryi*. The anterior end is prolonged into a partly eversible or retractile epimerite, ectoplasmal in nature and clear and hyaline in appearance (Fig. 2, A, *ep*). The epimerite is lost later in the life of the organism. The posterior end is narrower than the anterior, the general shape being falciform, with a distinct curvature (Fig. 2, A). The trophozoite is motile, its movements being chiefly of flexion.

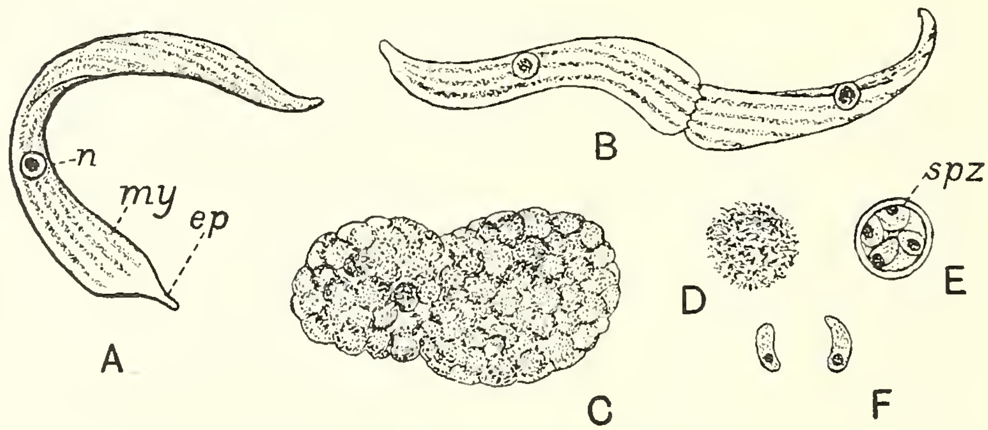


Fig. 2. *Selenidium echinatum*, after Caullery and Mesnil (1899).
- A. Free trophozoite, *ep* = epimerite, *n* = nucleus, *my* = myonemes.
- B. Two gametocytes in association.
- C. Cysts full of sporoblasts or young sporocysts, from a fresh preparation.
- D. Spherical sporocyst showing spiny exterior (epispore).
- E. Transverse section of sporocyst, with tetrazoic sporozoites, *spz*.
- F. Two free sporozoites.

The ectoplasm exhibits a series of fine, longitudinal, contractile striae, the myonemes (Fig. 2, A, *my*), which traverse the entire length of the body. Often 20 such myonemes are present, but the number varies for different species of *Selenidium* and may be smaller, *e.g.* four. The endoplasm is highly granular; little endoplasm is present in the epimerite region and it is less abundant at the posterior end of the body.

The nucleus is large and in a few species (*e.g. S. echinatum*) it is nearly spherical in shape (Fig. 2). In most species the nucleus is elongate oval, its long diameter being directed transversely with regard to the long axis of the animal (Fig. 3, II, VI). The nuclear membrane is very slightly marked, and the nucleus is filled with nuclear sap, somewhat granular in character, which surrounds the large laterally placed *karyosome*.

Intra-epithelial schizogony occurs in the *Selenidiidae*, and the intracellular stages are of long duration. The young schizonts in *S. caulleryi* resemble young trophozoites at first in being bluntly vermiform (Fig. 3, III), but they become more oval as growth proceeds. When they attain a length of about 50μ, nuclear fragmentation occurs (Fig. 3, IV), and the schizonts show 200 to 300 small, rounded nuclei scattered evenly through the cytoplasm, which does not seem to change. The cytoplasm next collects around each nuclear mass, and soon groups of small, curved, uninucleate merozoites are produced (Fig. 3, V). Each merozoite (in *S. caulleryi*) is 10μ to 12μ long and is motile.

The sporogony of *S. caulleryi* is not known. That of *S. echinatum* (from *Dodecaceria concharum*), as described by Caullery and Mesnil, (1899) may therefore be considered. In this case the two free

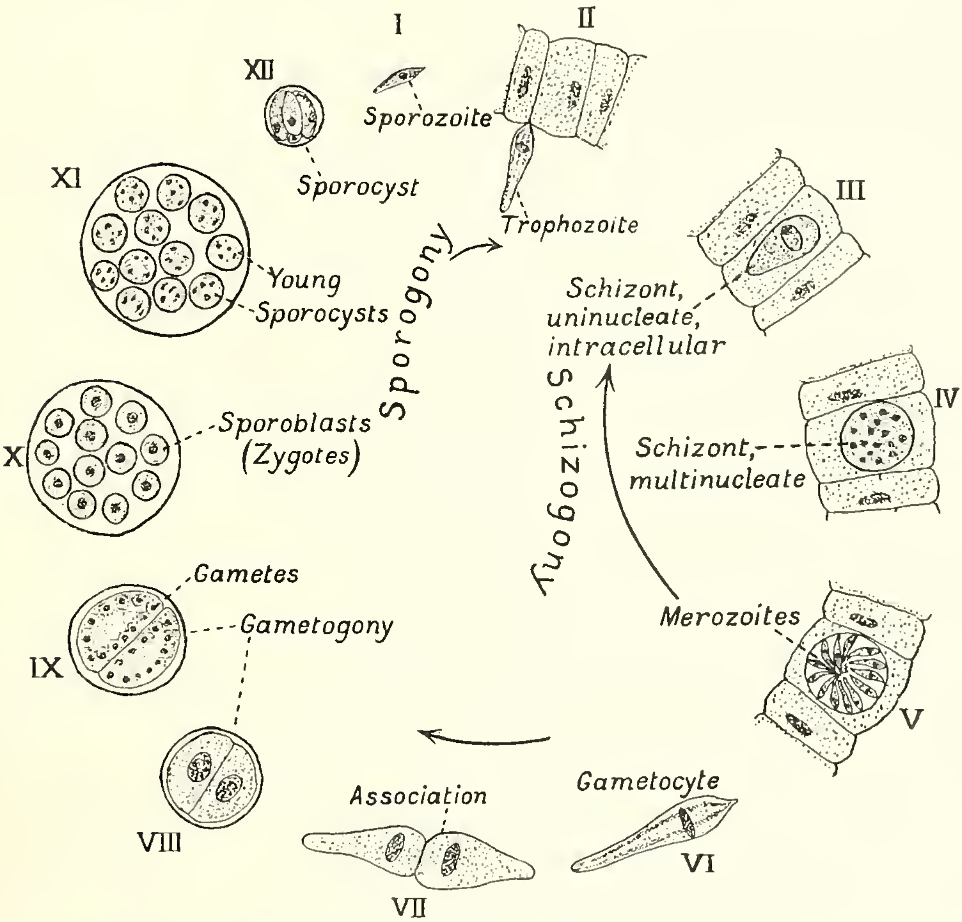


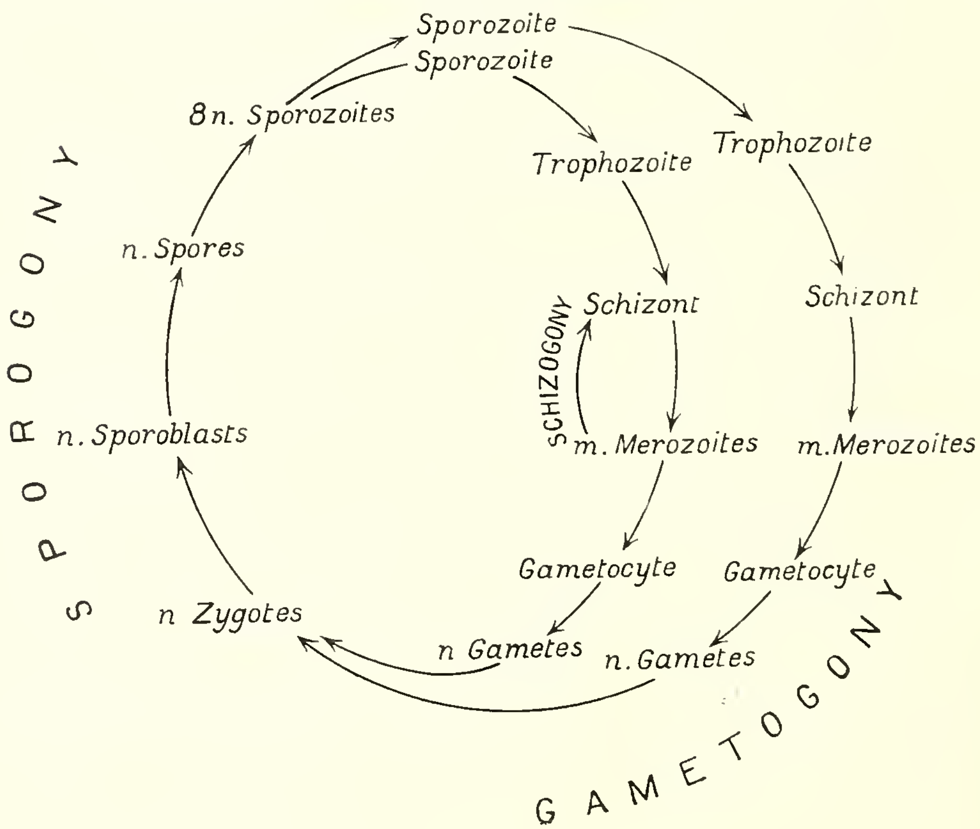
Fig. 3. Diagram of the life-cycle of *Selenidium*, based on that of *S. caulleryi* (Brasil) and *S. echinatum* (Caullery and Mesnil). Original diagram (H. B. F.). III—V, schizogony. VI—IX, gametogony. X—XII, sporogony.

trophozoites, which are at this stage gametocytes (Fig. 3, VII), associate at the ends corresponding to their epimerites (Fig. 2, B). Each gametocyte rounds itself off, and a common cyst is formed around them (Fig. 3, VIII). The nucleus of each gametocyte fragments and gamete nuclei are produced (Fig. 3, IX). Conjugation takes place between pairs of gametes and *sporoblasts* are produced (Fig. 3, X; also Fig. 2, C).

The sporoblasts give rise to spherical *sporocysts* (Fig. 2, D; Fig. 3, XI, XII) which are 8μ to 10μ in diameter. The cyst of the sporocyst in *S. echinatum* is finely spined (Fig. 2, D). Each sporocyst contains four symmetrically arranged sporozoites (Fig. 2, E; Fig. 3, XII). The small number of sporozoites in the sporocyst is most unusual among Gregarines, and, as Caullery and Mesnil point out, recalls the Coccidia. The spherical shape of the sporocyst is also unusual.

V. General Life-History.

The foregoing description and figures (Figs. 1, 3) will serve as outlines for the study of the Schizogregarine life-cycle, which I have set forth schematically in the following diagram.



In this diagram m refers to the variable number of merozoites formed from a schizont. Similarly, n refers to the large number of gametes formed from each gametocyte.

Each spore usually contains eight sporozoites.

The following points should be considered in connection with the above generalised diagram of the life-cycle:

Four sporozoites are present in *Selenidium* (*e.g. S. echinatum*), according to Caullery and Mesnil (see Fig. 2, E).

Intracellular schizogony prevails in *Selenidium* and *Merogregarina*.

Extracellular schizogony occurs in *Ophryocystis* and *Schizocystis*.

Nuclear reduction, so that one spore only is formed, takes place in *Ophryocystis*, this being expressed by the formula $n = 1$ (see Fig. 4, D).

In the *Aggregatidae*, the schizogony takes place in one host (Crab), and the sporogony in another (Cephalopod Mollusc).

The number of sporozoites in the spore varies in the different species of *Aggregata* (*e.g.* 3, 4, 8, 16, 24).

VI. DETAILED MORPHOLOGIES AND LIFE-HISTORIES.

The Schizogregarines at present comprise five well-marked families: *Ophryocystidae, Schizocystidae, Selenidiidae, Merogregarinidae* and *Aggregatidae.* The morphology and life-history of members of each of these families may now be set forth in some detail, avoiding, as far as possible, repetition of what has been written in the preceding section. It will be most convenient to consider these matters under the headings of the generic names, excepting in the case of the *Selenidiidae*.

(1) Genus **Ophryocystis**.

Regarding the genus *Ophryocystis* Schneider, an account of the life-cycle of which has already been given, the following further points are of importance.

The presence of cytoplasmic processes or fixative filaments in the extracellular or trophic forms is not entirely without parallel in the Eugregarines, for in the genus *Pterocephalus* (Schneider), a Eugregarine from the digestive tract of *Scolopendra cingulata*, similar processes of a fixative nature are known, proceeding from the epimerite region of the trophozoite of *Pterocephalus* into the cells of the epithelium of the gut of the host. In *Ophryocystis* the various forms of trophozoite or

schizont are fixed by their anterior ends to the epithelial cells of the Malpighian tubules of the Coleopteran host.

As regards the action of these fixative processes and of the parasite generally on the host, Léger considers that hypertrophy of the cytoplasm of the host cells may ensue, or that atrophy may occur, following perhaps upon hypertrophy. Granules of pigment are often seen in the cells in the parasitised area.

Two forms of schizonts, first distinguished by Léger (1907), have been mentioned as occurring in *Ophryocystis*. These forms are: (i) **mycetoid schizonts**, "multinucleate" in character (Fig. 4, A), which may be of irregular form, containing many nuclei closely packed (as seen in *O. hessei*), and (ii) **gregarinoid schizonts** with a well-defined contour and with fewer nuclei ("paucinucleate" of Léger), some 2 to 6 in number, as seen in *O. caulleryi* and *O. hessei* (Fig. 1, VI, VII).

The gregarinoid schizonts were the only forms known until recently (1907). They are the more commonly occurring forms. Léger considers that these paucinucleate forms represent "la vraie forme grégarinienne de l'*Ophryocystis*." The gregarinoid schizonts are the only ones which give rise to gametocytes ("gamontes" of Léger)[1] or sexual individuals (Fig. 4, B).

The mycetoid schizonts only give rise to merozoites ("schizozoites" of Léger) (Fig. 4, A), and so provide for the phases of schizogony. The daughter schizonts are formed either by (*a*) **plasmotomy**, wherein multinucleate portions or buds are constricted off, especially as in *O. hagenmuelleri*, which possesses branching schizonts, or (*b*) **schizogony**, as in the Coccidia, where the maternal cytoplasm breaks up and collects around each of the daughter nuclei, giving rise to uninucleate merozoites.

Mycetoid schizonts, at present, are only known to occur in the following species: *O. hessei*, *O. duboscqi*, *O. hagenmuelleri*, and possibly

[1] In describing *Ophryocystis* I have not used the terminology of Léger (1907), preferring to adhere to the older terminology, as used by Minchin (1903, p. 210), and introduced by Schaudinn more especially for the Coccidia. Léger used the term "gamonte" for a sexual individual or gametocyte. Although I admire Léger's work, and respect his unique knowledge of the Schizogregarines, the use of the term "gamonte" in this connection appears to me to be superfluous.

Léger's term "schizozoites," for the daughter forms resulting from schizogony (asexual multiplication or endogony) is obviously preferable to Schaudinn's term "merozoites." However, as the latter term is now well-established in the literature of the Parasitic Protozoa I have retained it. In view of the confusion already existing it appears undesirable to multiply technical terms. Wherever possible the terms in general use should be retained.

also in *O. francisci* and *O. buetschlii,* wherein schizonts containing about ten nuclei may be seen.

The phenomena of nuclear division in the gametocytes ("gamontes") during association (Fig. 4, C) are most interesting and significant. The single nucleus of the young globular gametocyte divides into two, giving rise to a "germinative nucleus" and a "somatic nucleus"

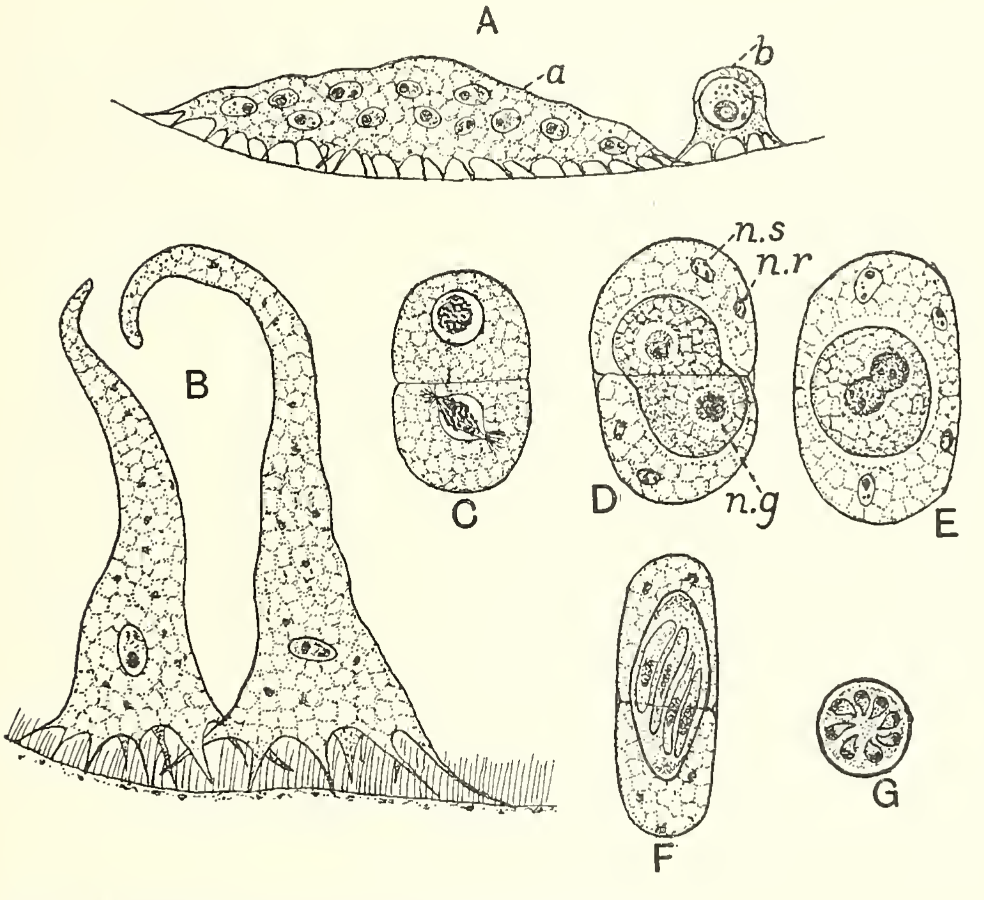


Fig. 4. *Ophryocystis*, various species, after Léger (1907).

A. *O. hagenmuelleri.* Mycetoid schizont (*a*) at the beginning of schizogony. (*b*) Merozoite, already somewhat grown.

B. *O. schneideri.* Gregarinoid gametocytes, with single nucleus, still attached to the epithelium of the host.

C. *O. hessei.* Gametocytes in association.

D. *O. mesnili.* Gametes in conjugation. *nr* = reduction nucleus, *ns* = somatic nucleus, *ng* = sexual nucleus (pronucleus).

E. *O. mesnili.* Zygote, with pronuclei still distinct.

F. *O. schneideri.* Cyst with ripe sporocyst. Falciform sporozoites represented inside.

G. *O. schneideri.* Transverse section of sporocyst showing octozoic spores.

(Fig. 4, D, *ns*). The latter gradually degenerates. We have then formed in each gametocyte a somatic portion and a sexual or gametic portion. The germinative nucleus itself next undergoes division into two, which Léger considers to be a "reducing division." Two small nuclei are thus formed, respectively styled the "sexual nucleus" and the "reduction nucleus" (Fig. 4, D, *ng*, *nr*). The sexual nucleus is a pronucleus, *i.e.* the nucleus of the gamete proper. We have illustrated here an interesting case of the maturation of the gametes, with reduction and degeneration of all except one from each gametocyte.

Parthenogenesis, such as the origin of a sporocyst in each gametocyte of an associating pair, or the production of one sporocyst by one gametocyte the partner of which is sterile, is known to occur in *Ophryocystis.* Normally, copulation of two isogametes, as described in the foregoing section (p. 375), produces a single sporocyst. Inside the sporocyst eight sporozoites usually occur (Fig. 4, F, G).

(2) Genus **Eleutheroschizon.**

Eleutheroschizon duboscqi Brasil (1906) is a parasite of the gut of *Scoloplos armiger* Oerst (= *Aricia muelleri* Rathke) and was first described by Brasil. It occurs fixed to the epithelium or free in the lumen of the gut. The fixed individuals are about 30μ long, and are dome- or bell-shaped. Their protoplasm is highly alveolar and their bases lie in hollows in the gut of the host. At the free extremity there is a cap with marked affinities for chromatin stains (Fig. 5, A, *chr*). The base is lobed, thus affording a superficial resemblance to *Ophryocystis.*

The schizogony only is well known. The merozoites are claviform and about 2μ to 5μ long. They glide between the cilia of the gut epithelium, and then penetrate for about half their length into the cells. They are never completely intracellular. Each merozoite grows to about 8μ in length. The part of it containing the large, vesicular nucleus with its prominent karyosome is internal, the rest external. When the organism is about 10μ long, an apical point appears, into which the nucleus migrates. The parasite may merely continue to grow and remain uninucleate or it may develop into a schizont (Fig. 5, B). The schizogony is extracellular.

When the parasite has attained the size of 15μ to 20μ, schizonts are formed, and nuclear division begins. A succession of such nuclear divisions occur, and the nuclei seem to be situated on bands of undulating protoplasm (Fig. 5, B). The nuclei finally reach the

periphery, and are extremely numerous and very small (Fig. 5, C). The protoplasm gathers around each nucleus, thus giving rise to a number of merozoites, irregularly arranged, and surrounding a mass of highly vacuolated, residual protoplasm (Fig. 5, D). Sporogony has never been seen, but it is probable that the uninucleate forms are gametes.

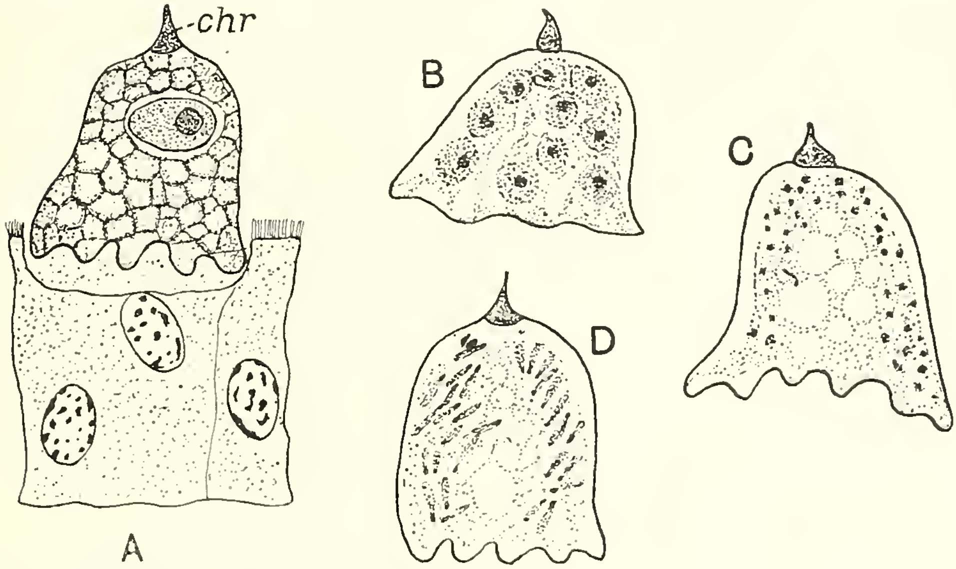


Fig. 5. *Eleutheroschizon duboscqi*, after Brasil (1906).

A. Uninucleate schizont (trophozoite), fixed to the gut-epithelium of the host. *chr* = chromatic cap.

B. Nucleus of schizont in process of division, the daughter nuclei situate on undulating cytoplasmic bands.

C. Further stage in nuclear division. Protoplasmic bands more separated. Vacuoles present in residual protoplasm.

D. Fully differentiated merozoites, lying near the periphery, away from the masses of highly vacuolated residual protoplasm.

(3) Genus **Schizocystis**.

No figures have yet been published of *Schizocystis gregarinoides*, of which an account has been given on p. 375. The absence of figures is unfortunate, but Léger, the discoverer of this interesting and unique organism, promises a paper thereon at an early date. Through the kindness of Professor Léger I have been able to examine some sketches of *Schizocystis*. I was glad to note the likeness in body-form of the multinucleate trophozoite (schizont) to that of *Siedleckia* (Fig. 6). In each organism there is concurrent increase in the number of nuclei and in the size of the schizont. The merozoites, which are large,

claviform and uninucleate, grow without change of form into gametocytes. The gametocytes associate in pairs, encyst, and form isogamous gametes, which conjugate in pairs and give rise to many sporocysts, biconical in shape and peripherally arranged inside the gametocyst. The spore-formation therefore takes place on the plan generally prevailing among the Gregarines.

(4) Genus **Siedleckia**.

The genus *Siedleckia* Caullery and Mesnil (1898) was created for *Siedleckia nematoides*, a somewhat aberrant parasite inhabiting the digestive tract of the Polychaetes, *Scoloplos muelleri* and *Aricia latreillei*[1]. The discoverers have noted its resemblance to verniform Schizogregarines like *Selenidium*. It is also very like *Schizocystis* in appearance, for it is nematoid in shape, and it is multinucleate. It has a similar habitat to *Selenidium*, and performs similar movements. The parasite, as stated, is a small vermicular organism, of which one extremity is fixed to the wall of the gut and is immobile, while the other or distal extremity is free, and executes vigorous helicoid movements of torsion and flexion.

The size of *Siedleckia* varies, specimens ranging in length from 8μ to 150μ. While they are very transparent, their protoplasm is granular. In life, clear areas are seen at intervals. These are the nuclei, which appear to greater advantage in stained preparations. The young forms are somewhat spindle-shaped and have few nuclei (Fig. 6, A); as they increase in size nuclear multiplication occurs (Fig. 6, B, C). The nuclei at first lie one behind the other in a single row (Fig. 6, B, C, D), but as they increase in numbers the order is broken and several rows may be seen (Fig. 6, E). This nuclear increase is far more noticeable in the distal or free end, which is rounded, than in the proximal portion (Fig. 6, E), which may be attached to the cells of the host. When the number of nuclei reaches about 120, asexual reproduction commences.

The parasite is now about 150μ long, and contains a relatively large number of nuclei. Its growth still continues, but at the same time protoplasm collects around certain peripheral nuclei at the distal end, and these become successively constricted off (Fig. 6, F) as small, spherical masses, each with a very small number of nuclei (Fig. 6, G). Each of these buds probably can develop into a new *Siedleckia*

[1] Dobell (*Q. J. M. S.*, Jan. 1909) found *Siedleckia* in *Aricia fœtida*.

(Fig. 6, H). This method of reproduction, which is a form of schizogony, is comparable to the simple plasmotomy (Doflein) occurring in the *Neosporidia*. Up to the present this is the sole form of reproduction that has been described; the sporogony and mode of infection of the host are unknown. It is possible that resistant forms of *Siedleckia* exist, as Caullery and Mesnil state that they only obtained their material at a particular period of the year (August and September). Probably if the hosts were searched at their time of reproduction, the resistant phases of the parasite would be found.

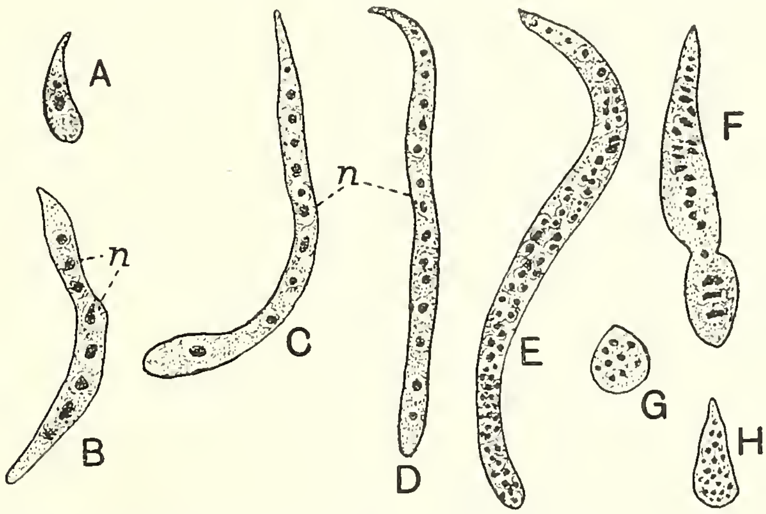


Fig. 6. *Siedleckia nematoides*, after Caullery and Mesnil (1899).

A. Young trophozoite with two nuclei.
B. Older form, with nuclei (*n*) in a single row.
C, D. Trophozoites still older, nuclei in single file.
E. Full-grown parasite. Nuclei in a single row at the more pointed end, in two or more rows at the distal or rounded end.
F. Parasite showing small multinucleate portions constricting off, by plasmotomy.
G. Young, somewhat spheroidal form, produced by constricting off the parent, as in F.
H. Older stage of a form, such as shown in G, which is now growing into a vermiform trophozoite.

The systematic position of *Siedleckia* still remains undetermined. The general appearance and asexual multiplication by plasmotomy are certainly most suggestive of what has been described by Léger (1900) for *Schizocystis*, while certain of Léger's sketches (see p. 383) of *Schizocystis* further support the view that there is probably some affinity between *Siedleckia* and *Schizocystis*. Caullery and Mesnil (1899), who described *Siedleckia*, noted its superficial resemblance to the

Selenidiidae, while they also saw some analogy with *Amoebidium.* Inasmuch as the protozoal nature of the latter organism has now been refuted, while *Siedleckia* is undoubtedly a Protozoon, the comparison with *Amoebidium* cannot stand. On the whole, it would be well to place *Siedleckia* and *Schizocystis* in one group, as Brasil (1907) has done, a course that has been followed by Léger and Duboscq, uniting them on account of their similarity of habitat, their resemblance to one another when undergoing nuclear multiplication, and their method of formation of daughter forms containing one or more nuclei.

The multinucleate condition of the trophozoite, forming a so-called "plasmodial" stage, has led Caullery and Mesnil (1905) to discuss *Siedleckia* in their excellent paper on the *Haplosporidia.* Until something is known as to the existence or otherwise of sporogony, the definite systematic position of *Siedleckia* cannot be fixed. In the preparations which I have seen, a structure of the nature of an epimerite seems to exist, and this feature would accentuate the gregarine-like character of the parasite.

(5) Family **Selenidiidae.**

There is at present one well-defined genus in this family, viz. the genus *Selenidium.* This genus is, however, somewhat difficult to define from the point of view of a complete life-cycle. The species placed therein, by Labbé (1899) (under the name *Polyrhabdina*), are in some cases very doubtful members of the genus. The type species is *Selenidium pendula* Giard (1884), originally described as from the body cavity of *Nerine* sp. *S. pendula* possesses longitudinal myonemes. The habitat of this parasite as given by Giard is incorrect, for Caullery and Mesnil (1899) re-discovered it in the digestive tract of *Nerine cirratulus.* Selenids were undoubtedly seen earlier (*e.g.* Kölliker 1845), but were usually considered to be Nematode embryos.

Mingazzini (1891) described Selenid forms, since they possessed longitudinally disposed myonemes, under the generic names of *Polyrhabdina* and *Esarhabdina,* according to the large or small number of the myonemes present. In 1892 Léger described trophic phases of Gregarines, which he placed in a new genus *Platycystis,* possessing myonemes longitudinally arranged, in some cases with a spiral twist.

In 1898 Caullery and Mesnil contributed a very important paper on a Gregarine exhibiting a schizogonic phase in its life-cycle. The

host under investigation was the Polychaete worm *Dodecaceria concharum,* and it contained two parasites, one in the coelom known as *Gonospora longissima*, and the other in the gut, known as *Selenidium echinatum.* Caullery and Mesnil apportioned the schizogony, which occurred in the gut epithelium, to the former (*Gonospora*). Brasil (1907, pp. 389—393) discussed the matter at length, and considered that the schizonts really formed a part of the life-cycle of *S. echinatum.* Intra-epithelial schizogony also occurs in the *Selenidium* described by Caullery and Mesnil from the gut of *Scololepis fuliginosa.*

The most detailed account of schizogony in a *Selenidium* is that given by Brasil (1907) for *S. caulleryi*, from the gut of the Polychaete, *Protula tubularia.* Brasil's researches on *S. caulleryi* have been summarised in the preceding section (see pp. 376, 377, and Fig. 3, III, IV, V).

Further researches on the schizogony of Selenids were published by Brasil and Fantham (1907) who studied species found in the gut of Phascolosomes (*Phascolosoma vulgare* and *P. elongatum*). Two species of *Selenidiidae* occur in these Gephyreans; the species are differentiated at present by the number of longitudinal myonemes occurring in the respective trophozoites (Fig. 7). In the **first species** (α), which is rectangular in section, each face has only some two or three myonemes (Fig. 7, A, B) which are broad and very apparent. Two forms are recognised in this species, one with elongate trophozoites (Fig. 7, A), whose breadth is about one-fifteenth of its length, and which may reach 350μ in length, and the other, a shorter, stumpy form, whose breadth is about one-third of its length (Fig. 7, B). The nucleus of the elongate forms is nearly spherical (Fig. 7, A), that of the stumpy form is transversely ovoid (Fig. 7, B). Intermediate forms, however, occur between these elongate and stumpy types.

The **second species** (β) possesses many fine, longitudinal myonemes (Fig. 7, C, D), some 30 to 40 in number, while the body is circular in section. Elongate (Fig. 7, C) and stumpy (Fig. 7, D) types occur, but the differences in length between them are not so marked, and the relation of the breadth to the length is never less than one-eighth.

Schizogony occurs in the epithelium of the gut of the Phascolosomes. In the deeper parts of the gut epithelium, the schizonts form oval cysts which project slightly into the coelom. Each schizont gives rise to some 30 to 40 merozoites measuring about 12μ in length. Lateral association has been seen between the Selenids with fine myonemes (Fig. 7, E).

Dogiel (1907) has described a *Selenidium* from the gut of *Sipunculus nudus,* which he places, not in the genus *Selenidium,* but in that of *Schizocystis,* as *Schizocystis sipunculi.* Dogiel's parasite is very probably

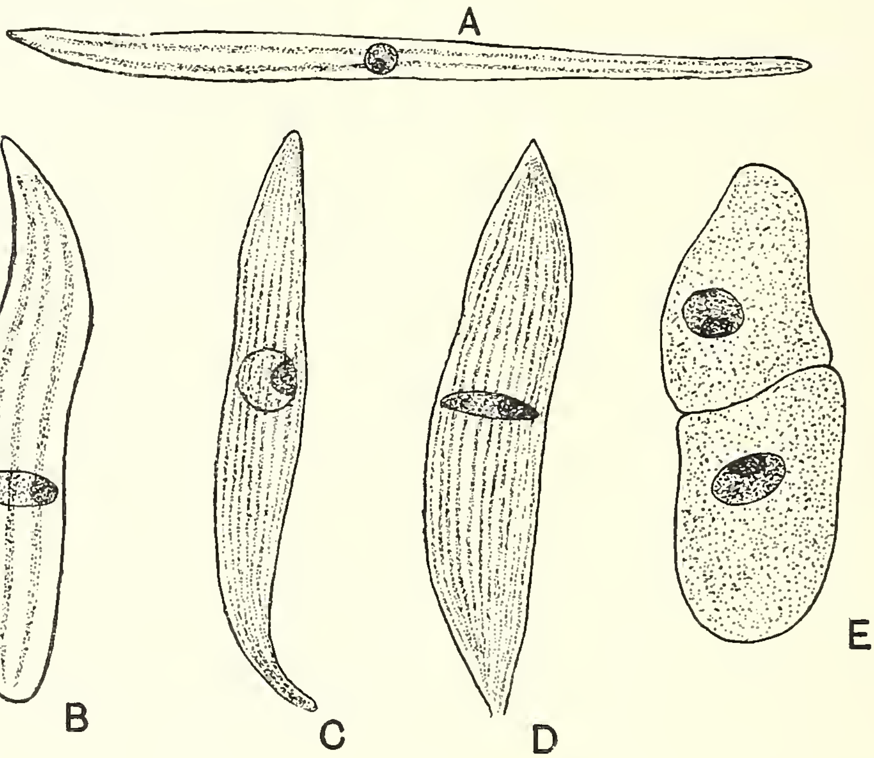


Fig. 7. *Selenidiidae,* described by Brasil and Fantham (1907), from the gut of Phascolosomes (*Phascolosoma vulgare* and *P. elongatum*). Original figures (H. B. F.), copied from drawings made with camera lucida. × 600 (except A which is × 300).

A. Species (α), trophozoite, with few myonemes, elongate in form, and with rounded nucleus.
B. Species (α), stumpy form, nucleus transversely ovoid.
C. Species (β), with many myonemes, slender trophozoite with rounded nucleus.
D. Species (β), stumpy form, with transversely ovoid nucleus.
E. Species (β), association of gametocytes.

a species of *Selenidium,* it is certainly a member of the family *Selenidiidae,* and is best termed, at present, *Selenidium sipunculi.* Dogiel's account of schizogony therein is apparently inaccurate (see Brasil and Fantham, 1907).

While working at Banyuls (Pyrénées Orientales) in the spring of this year (1908) I found similar Selenid parasites in the Terebellid, *Polycirrus aurantiacus.*

The shape of the epimerite in the trophozoite of *Selenidium* is variable. Caullery and Mesnil (1899) describe two forms of Selenid trophozoites with epimerites from *Cirratulus cirratus*: (i) a comma-shaped variety (S. "en virgule"), and (ii) a semi-colon-like variety (S. "en point et virgule"). This variation is discussed by Brasil (1907), who attributes the truncated aspect to an invagination of the anterior pointed region (Brasil (1907), pp. 376, 384, 385).

Apart from the possibly injurious effect of the parasite upon the host cell, due to the organellae which serve for attachment, the investigations of Brasil (1907, Fig. 14) have shown that the merozoites of *S. caulleryi* may issue in masses into the lumen of the gut, thus displacing the epithelium.

It is interesting to note that species of *Selenidium* may in turn be parasitised. The Selenids of Phascolosomes (Brasil and Fantham, 1907) as well as certain species of *Selenidium* in Polychaetes (Caullery and Mesnil), and the *Platycystis* (= *Selenidium*) of Léger in *Audouinia* all contain minute parasites, one stage of which has the characteristic morula-form (cf. *Chytridiopsis* of Aimé Schneider).

In the following section an attempt has been made to set forth a complete list of the various species of *Selenidium* (see p. 399). This has entailed much labour, and the searching of many papers and figures. Of the lists of hosts of *Selenidium* given by Labbé (1899, who retains Mingazzini's name *Polyrhabdina*) and Minchin (1903), two members must certainly be radically revised as regards harbouring parasites belonging to the genus *Selenidium*. Regarding one of these, that described by Greeff (1885) as *Gregarina annulata* from the intestine of *Rhynchonerella fulgens*, it must be noted that it is transversely annulate as figured in the original, and seems to have been referred incorrectly to Mingazzini's genus *Polyrhabdina*. As at present understood, and pending further researches, Greeff's parasite has no place in the genus *Selenidium*. The other parasite, described by Ray Lankester (1866) as *Monocystis eunicae*, has been referred by Labbé to the so-called genus *Polyrhabdina*. It was found in the gut of *Eunice harassei*, and is listed by Minchin in the genus *Selenidium*. Reference to Lankester's figure does not support the view that "*Monocystis eunicae*" belongs to the genus *Selenidium*, and the discoverer makes no mention of longitudinal striations being present. Pending further researches it should certainly be removed from the genus *Selenidium*.

Most of the species at present placed in the genus *Selenidium* are only known in their trophozoite phase; further researches are greatly

needed on schizogony and sporogony. The sporogonic stages should be looked for especially at the time of reproduction of the Annelid hosts. Sections should be made of the digestive tracts of parasitised hosts for stages of schizogony of the parasite.

(6) Genus **Merogregarina.**

A new Schizogregarine from the alimentary tract of a Protochordate, namely, the Composite Ascidian, *Amaroucium* sp. has recently been described. The host came from New South Wales. The parasite belongs to a new genus, and Miss Annie Porter, who discovered it in 1908, has given it the name *Merogregarina amaroucii* (Fig. 8). The trophozoites occur in fair abundance in the gut of the host, and in some places a very heavy infection occurs in the lumen of the gut. The free trophozoites are 23μ to 31μ long and from 11μ to 15μ broad (Fig. 8, A, B). They are non-septate or monocystid. An epimerite about 4μ to 6μ long is present and it is shaped like a lance-head (Fig. 8, A, *ep*). Each trophozoite possesses a well-defined cuticle, a clear ectoplasm and a granular endoplasm. Myonemes are seen in the

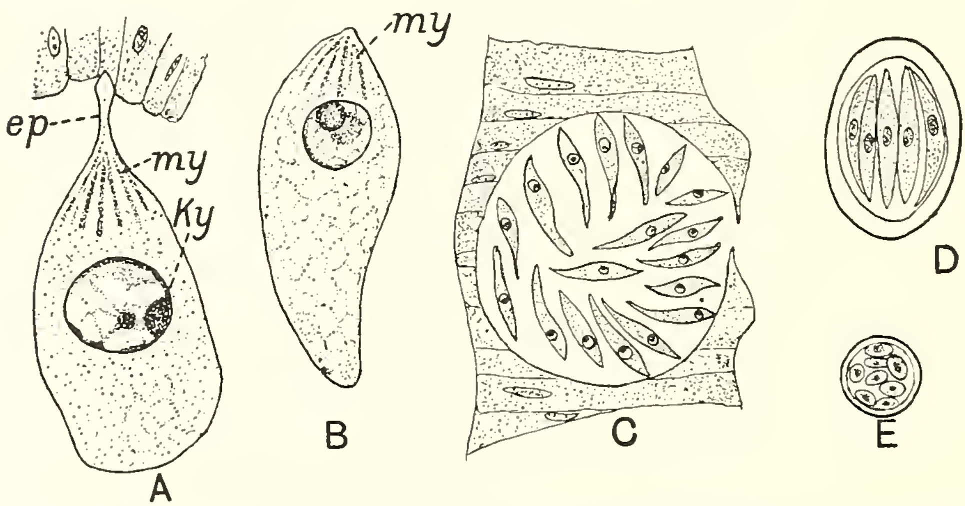


Fig. 8. *Merogregarina amaroucii* Porter (1908). Original drawings by Miss Annie Porter.
- A. Trophozoite with lance-shaped epimerite (*ep*). Myonemes (*my*) in pre-nuclear region. Large nucleus with marked karyosome (*ky*), and plasmosomes.
- B. Trophozoite whose epimerite is lost. Karyosome somewhat dumb-bell shaped.
- C. Section of gut of *Amaroucium* sp. showing group of merozoites cut longitudinally. The channel by which they issue singly into the lumen of the gut is here seen to be open.
- D. Sporocyst in longitudinal section, showing sporozoites.
- E. Sporocyst in transverse section, showing eight sporozoites cut across.

anterior region (Fig. 8, A, B, *my*), stretching from the epimerite to the neighbourhood of the nucleus in a fan-like manner. The nucleus is large and well-marked. It is vesicular, with a large karyosome (Fig. 8, A, *ky*) lying in a faint, chromatic reticulum. The karyosome may be spherical, notched or dumb-bell shaped (Fig. 8, B). One or more plasmosomes are frequently to be seen lying in the nuclear sap. There is a definite nuclear membrane with an irregular lining of chromatin internally.

The asexual cycle, or endogenous multiplication, is completely known. Schizonts occur within the gut epithelium of the Ascidian host. These schizonts are about 17μ long by $10{\cdot}5\mu$ broad. When they have attained this size the schizonts begin to sporulate. The nucleus of a schizont divides into 8 to 18 fragments of chromatin, there being no remains of the parent nucleus. The daughter nuclei are distributed fairly evenly throughout the mother cell (schizont). The cytoplasm of the latter collects around each daughter nucleus and so a small, but apparently inconstant, number of merozoites is formed (Fig. 8, C). These separate and find their way into the lumen of the gut and so account for auto-infection of the host.

Sporogony is also known, though somewhat incompletely. Two fully-formed trophozoites or gametocytes come together, associate and encyst. Finally sporocysts 14μ by 11μ are seen (Fig. 8, D). The sporocysts are oval and contain eight small sporozoites (Fig. 8, E) arranged "en barillet." These sporozoites serve for cross-infection.

Merogregarina reacts on its host, for trophozoites, while still in the gut lumen, are seen lying in bays or depressions in the epithelium of the gut. The effect of the schizont is, of course, more marked, and is of the usual character associated with intracellular schizonts.

Merogregarina is considered to be nearest the *Selenidiidae* in affinities, though it has octozoic spores, and the myonemes are only seen in the anterior region of the trophozoite.

This most interesting parasite, *M. amaroucii*, extends the distribution of the Schizogregarines to the *Protochordata*. It is also of interest to note that *Merogregarina* harbours a *Chytridiopsis*-like parasite within its protoplasm[1]. In this respect it resembles various *Selenidiidae*, as described by Brasil and Fantham, Caullery and Mesnil, and Léger (noted on p. 389).

[1] For further details see Porter (1908); a complete paper, fully illustrated, will appear in due course.

(7) Genus **Aggregata**.

The genus *Aggregata* was founded by Frenzel (1885) for Gregarines infesting various Decapod Crustacea (*e.g. Portunus arcuatus* and *Carcinus maenas*). These Gregarines in Crustacea had been seen by many of the earlier writers, *e.g.* by Cavolini (1787), Rudolphi (1819), and Diesing (1851). Frenzel considered that the coelomic cysts, really occurring in the peri-intestinal lymphoid tissue and projecting into the haemocoel of the crabs, belonged to the life-cycle of the Gregarines found in the lumen of the intestine of the same hosts, and that they arose from a conjugation. Labbé (1899) accepted Frenzel's genus and united it with *Porospora* of the lobster under the *Gregarina gymnosporea*, since naked sporozoites (so-called) occurred, grouped around residual protoplasm.

Geoffrey Smith (1905), however, showed that no conjugation occurred in the life-history of the "coelomic" Gregarine, *Aggregata inachi*, of *Inachus dorsettensis*. He found that at the beginning of sporulation there was only a single nucleus which divided and gave rise to daughter nuclei at the periphery of the cyst. These nuclei, each surrounded by a small mass of protoplasm, became so-called sporozoites. The intestinal Gregarines of crabs and the coelomic cysts of *Aggregata* must, then, be separated, as has now been done by Léger and Duboscq. No resistant sporocysts are formed by *Aggregata* in the crab.

Regarding the development of the sporozoites of *Aggregata*, Frenzel (1885) suggested the remarkable hypothesis that their further development might occur in Cephalopods, for which the crabs serve as food. This interesting forecast was found to be correct by Léger and Duboscq (1906), who demonstrated experimentally that the *Aggregata* of crabs and the *Eucoccidium* of Cephalopods only represent different stages in the life-cycle of one parasite. In this connection it is interesting to note that the gastric juice of *Sepia* is without action on the sporocysts of *Aggregata*. From the researches of Moroff and Léger and Duboscq it is now considered that we have in the parasite in question a *digenetic* and *heteroïc* Gregarine, whose schizogony occurs as "*Aggregata*" in the crab, and whose sporogony occurs as "*Eucoccidium*" in the Cephalopod.

Eucoccidium was formerly unique among the *Coccidia* in possessing no schizogonous cycle, a sporogonic one only being known. The stages in the Cephalopods had been investigated by many well-known workers in the past, including Lieberkühn (1854—5), Eberth (1862), Schneider

(1875), Mingazzini (1892), Labbé (1896), Siedlecki (1898), and others. The work of Siedlecki is especially noteworthy. Species of *Eucoccidium* are known in various cuttlefish and *Octopus*. The multiplicity of species in Cephalopods, and of workers thereon, has led to great confusion in the nomenclature. The so-called Coccidian genus was variously known as *Eucoccidium*, *Klossia*, *Benedenia*, *Légeria*, and *Légerina*.

Léger and Duboscq have now ended the confusion by giving the name *Aggregata* (*Eucoccidium*) *eberthi* to the parasite whose schizogony occurs in *Portunus depurator* at Cette and in *Portunus arcuatus* at Roscoff, with its sporogony in *Sepia officinalis*. The methods of Léger and Duboscq, as before mentioned, were experimental. They fed specimens of *Portunus* on parasitised stomachs of *Sepia*. These stomachs contained sporocysts of *A. eberthi*. After a period varying from $1\frac{1}{2}$ to 36 hours in the crab's stomach, the ripe sporocysts opened by the bursting apart of the two valves (Fig. 9, B) and the sporozoites were set free in the intestinal juice of the crab. The sporocysts of *A. eberthi* (Fig. 9, A), which are about 9μ in diameter, contain three sporozoites (Fig. 9, A, B). The free sporozoites are vermicular, curved or **S**-shaped (Fig. 9, B), and have a very slightly enlarged anterior extremity, with an elongate nucleus about 5μ long near the posterior end, without a distinct karyosome. The sporozoites average 15μ to 18μ in length by $1{\cdot}8\mu$ to 2μ in breadth. When set free the sporozoites rapidly penetrate the epithelial cells of the intestine of the crab. Most of them traverse the epithelium and soon attain the basal membrane, through which they try to pass. This basal membrane, however, is thick and resistant, and many of the sporozoites are stopped by it. Some succeed in piercing the basal membrane and gain the peri-intestinal lymphoid layer, where their further development takes place. The other sporozoites, which are unable to penetrate the basal membrane, remain imprisoned in the crab's intestinal epithelium, and ultimately die; they hypertrophy, become pyriform and degenerate.

The sporozoites (Fig. 9, C), which have succeeded in reaching the lymphoid tissue, remain there and continue their development. For a time their primitive length (15μ to 18μ) is retained, while they increase in breadth (from 2μ to 8μ), becoming reniform and then nearly spherical (Fig. 9, D). The period of growth lasts about 10 days. During this growth in breadth, the nucleus changes in position, in shape and in structure. From elongate oval it gradually becomes spherical, and takes up a central position in the cytoplasm. The chromatin of the nucleus,

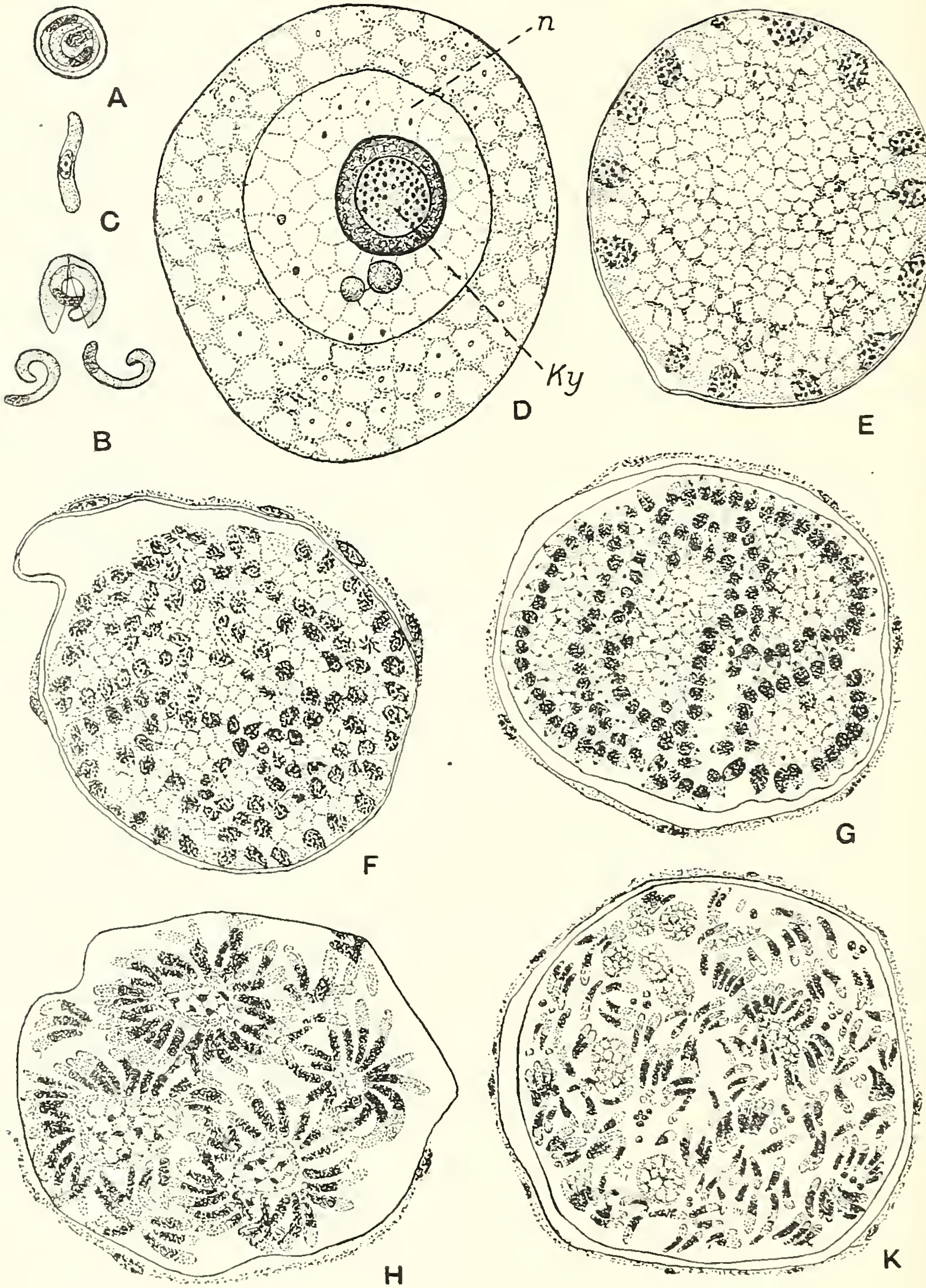

n
Ky
A
B
C
D
E
F
G
H
K

Fig. 9. *Aggregata eberthi* (Labbé). After Léger and Duboscq (1908).

A. Sporocyst from the stomach of *Sepia officinalis*. Three sporozoites inside.

B. Sporocyst showing valvular dehiscence, with two sporozoites free, the third still between the valves. Note posterior position of nucleus in the sporozoites. From gut of *Portunus*.

C. Sporozoite at beginning of growth, after its passage through the gut-wall of *Portunus*. Nucleus has changed its position and become central.

D. Further stage in growth; the parasite is now a young schizont. The nucleus (*n*) has become central, and the karyosome (*ky*) has attained its highest degree of complexity.

E. Schizont. Nuclear division and migration of small masses of nuclear material to the periphery. This and following stages are enclosed in a thin cyst in the peri-intestinal lymphoid layer, projecting into the haemocoel of *Portunus*.

F. Schizogony. Further nuclear multiplication.

G. Schizogony. End of nuclear multiplication; nuclei arranged along serpentiform strands of protoplasm. Commencement of differentiation of merozoites.

H. Merozoites (schizozoites) fully differentiated and arranged at the periphery of somewhat rounded masses of residual protoplasm.

K. Merozoites dispersed and lying free among masses of residual protoplasm.

at first arranged in the form of a reticulum, becomes peripheral and loses much of its capacity for staining. During this period a karyosome becomes evident (Fig. 9, D, *ky*) and gives off granules of chromatin into the cytoplasm. At this stage we have a young massive Gregarine provided with a spherical nucleus containing a large karyosome (Fig. 9, D), the organism being enclosed in a membranous cyst in the peri-intestinal lymphoid layer. The parasite is now a young schizont. The nucleus of the schizont, which possesses a nuclear membrane, complex karyosome and central reticulate zone (Fig. 9, D), then undergoes multiple division, giving rise to peripherally placed daughter nuclei (Fig. 9, E, F). These daughter nuclei are arranged along the edges of serpentiform, cytoplasmic islets produced by small invaginations of the mother cytoplasmic mass (Fig. 9, G). The daughter nuclei, with their cytoplasm collecting round them, give rise to rosettes of naked gymnospores or merozoites arranged around spherical masses of residual protoplasm (Fig 9, H, K).

It is of interest to note that Léger and Duboscq found two forms of schizont. One form has a thick membrane and grows to about 50μ in length at the rate, roughly, of 1μ per diem, attaining their full size in 40 days. These are regarded as male Gregarines. The other form has a thin membrane and grows more rapidly and may attain a diameter of 200μ in 45 days, at the rate roughly of 4μ a day. This is regarded as a female Gregarine. The cytoplasm of a Gregarine which has practically attained its greatest size contains certain granules some of which have staining reactions like those of chromatin, while there are others, which,

though they do not stain in the same manner, are probably derivatives of chromatin. Grains of paramylum are also present, scattered in the network. Complicated nuclear changes take place during the growth both of the schizont and gametocyte.

There are slight differences in the merozoites formed from the two kinds of schizonts. The length of the merozoites averages 10μ to 11μ; but slight differences occur in the breadth of the two varieties.

Léger and Duboscq remark that during the course of the development in the lymphoid tissue, a large number of young parasites are the prey of phagocytes which bring about their degeneration. This waste, together with the arrest of some of the sporozoites by the basal membrane, accounts for the rarity of cysts reaching maturity in nature.

Crabs do not feed directly on the stomachs of *Sepia* under natural conditions, but take up the sporocysts while eating the excrement of Cephalopods.

As regards the sporogonic stages of *Aggregata* in Cephalopods (which are initiated by merozoites from *Aggregata* of the crab), the most recent account is that of Moroff, who disagrees—especially in his preliminary communication in 1906—with the account given by Siedlecki in 1898. In his earlier papers Moroff has especially disputed Siedlecki's account of fertilisation. Moroff considers that the macrogametocyte forms more than one macrogamete, or that "fertilisation occurs at the time of formation of the [primary] sporoblasts," and that fertilisation occurs between anisogamous gametes, as in some Gregarines. However, Moroff (1908, p. 119) subsequently recognised that most of his earlier figures of fertilisation have nothing to do therewith, but merely represent stages in the division of the sporoblast. Moroff's grounds, then, for considering *Aggregata* as a Gregarine are insufficient (as remarked by Caullery in his review, *Bull. Inst. Pasteur*, VI. p. 723). New researches are needed on this important matter, and Siedlecki's descriptions of fertilisation are not yet refuted.

Moroff forms many new species of *Aggregata* from the parasites observed by him in an *Octopus* (sp ?) of Cavalière (Var, Mediterranean). The specific differences according to Moroff are based on nuclear structure (*e.g.* variation of karyosome), or on the size of the gametocytes. Probably the number of species will ultimately have to be reduced. The sporocysts of species of *Aggregata* in the *Octopus* contain 8 to 24 sporozoites. Of the older species, *A. eberthi* and *A. octopiana* (Schneider) are the chief. Moroff's paper (1908) is largely written from the physiological point of view.

To summarise: it should be noted that the *Aggregatidae* form a perfectly distinct family, somewhat apart from other members of the *Schizogregarinae*. They differ (i) in the absence of association between their gametocytes, which entails subsequent fertilisation between anisogamous free gametes, and (ii) in a change of host being necessary to complete their life-cycle.

Léger and Duboscq (1908, p. 98) point out that in some Decapod Crustacea there occur both intestinal and coelomic Gregarines, *e.g.* in *Pinnotheres pisum* and *Eupagurus prideauxi*. In others there occur only intestinal Gregarines, as in *Chthamalus, Phronima, Gammarus, Athanas,* while in *Inachus dorsettensis*, G. Smith (1905) saw only coelomic Gregarines. These Gregarines, found in two entirely different situations, are quite distinct, a fact which was overlooked before the researches of G. Smith in 1905 and Léger and Duboscq (1907). For the intestinal Gregarines of Decapods, Léger and Duboscq (1907) proposed the generic name *Frenzelina*. The members of the genus *Frenzelina* are typical Eugregarines belonging to the family *Clepsydrinidae*, and are entirely intestinal in habitat. The coelomic cysts found in the above-mentioned Decapods belong to *Aggregata*.

VII. Systematic.

In this section it will be convenient to define, as far as possible, the diagnostic characters of the various families of the Schizogregarines, and then to tabulate the genera and species contained therein, so far as known.

1. Family **Ophryocystidae,** Léger and Duboscq.

Schizonts extracellular, increasing simultaneously in volume and in number of nuclei.

Two genera: *Ophryocystis* and *Eleutheroschizon.*

(1) Genus *Ophryocystis*, A. Schneider.

Monocystid Schizogregarines with extracellular schizonts, conical in shape, fixed to the epithelium of the host by root-like cytoplasmic processes. Gametes isogamous. A single sporocyst, containing eight sporozoites, formed after conjugation.

Nine species are known from the Malpighian tubules of *Coleoptera* (especially *Tenebrionidae*).

Parasite	Habitat	Host	Remar
Ophryocystis buetschlii, A. Schneider	Malpighian tubules	*Blaps* sp. (? *B. mucronata*)	
O. francisci, A. Schneider	,, ,,	*Akis algeriana*, *A. acuminata*	Mycetoid schizont (?) known
O. schneideri, Léger and Hagenmüller	,, ,,	*Blaps magica*.	
O. hagenmuelleri, Léger	,, ,,	*Olocrates gibbus*	Mycetoid schizonts known
O. caulleryi, Léger	,, ,,	*Scaurus tristis*	
O. mesnili, Léger	,, ,,	*Tenebrio molitor*	
O. perezi, Léger	,, ,,	*Dendarus tristis*	
O. hessei, Léger	,, ,,	*Omophlus brevicollis*	Mycetoid schizont known
O. duboscqi, Léger	,, ,,	*Otiorhynchus meridionalis*, *O. ligustici*, *O. fuscipes*	Mycetoid schizont known

(2) Genus *Eleutheroschizon*, Brasil.

Schizogony extracellular. Sexual reproduction unknown. The general form of the trophozoite (multinucleate schizont) and its manner of attachment to the intestinal epithelium of the host recalls *Ophryocystis*. The trophozoite possesses a chromatic cap.

One species only known at present.

Parasite	Habitat	Host	Remarks
Eleutheroschizon duboscqi, Brasil	Intestine	*Aricia muelleri*, Rathke = *Scoloplos armiger*, Œrst.	Schizogony only known

2. Family **Schizocystidae**, Léger and Duboscq.

Trophozoites cylindrical, elongate or vermiform, with an anterior clearer region. Monocystid. Schizogony extracellular, with nuclear multiplication during growth.

Two genera: *Schizocystis, Siedleckia.*

(1) Genus *Schizocystis*, Léger.

Trophozoites vermiform, affixed to the epithelium of the host by the anterior, clear extremity. Gametes isogamous, which, after conjugation, produce numerous octozoic spores.

One species only known.

Parasite	Habitat	Host	Remarks
Schizocystis gregarinoides, Léger	Intestine	*Ceratopogon* sp., larva	From Lake Luitel, Alps

(2) Genus *Siedleckia*, Caullery and Mesnil.

Trophozoites vermiform. Schizogony, by plasmotomy, only known. Sporogony unknown.

One species.

Parasite	Habitat	Host
Siedleckia nematoides, Caull. and Mesn.	Gut	*Scoloplos muelleri*, *Aricia latreilli*, *Aricia fœtida* (Dobell)

3. Family **Selenidiidae**, Brasil.

Schizont intracellular, uninucleate during growth, becoming multinucleate at the end of its development. Trophozoites (gametocytes) free, vermiform, motile, with longitudinal myonemes.

One genus, *Selenidium*.

(1) Genus *Selenidium*, Giard.

This genus, as emended by Caullery and Mesnil in 1899, includes *Polyrhabdina*, Mingazzini (1891), *Esarhabdina*, Mingazzini (1891), and *Platycystis*, Léger (1892).

The epimerite may be slender and conical, or large and globular (Caullery and Mesnil, 1899). The latter form is probably due to invagination (Brasil, 1907). Spores spherical, spined and tetrazoic. Parasites of Annelids (Polychaetes and Gephyrea).

Many species, the majority of which are ill-defined, and need re-investigation, especially from the point of view of possible schizogonic stages.

The following list, which has entailed much labour, is the first of its kind for the genus; it is hoped that it is complete. It has been considered advisable to list the hosts in the first column, as so many of the species are unnamed. In view of our incomplete knowledge it appears inadvisable to supply names to such species of *Selenidium*.

Host	Habitat	Parasite	Remarks
Aricia sp.	Gut	*Selenidium sabellae*, Lank., 1863	
Audouinia filigera	,,	*S. cirratuli*, Lank., 1866	
Audouinia tentaculata	,,	*Selenidium* sp., C. & M.*, 1899	
Cirratulus cirratus (= *C. borealis*)	,,	*S. cirratuli*, Lank., 1866	Two species described by C. & M. (1899)
Ctenodrilus serratus	,,	*Selenidium* sp., C. & M., 1899	Small size

* C. & M. = Caullery and Mesnil.

Host	Habitat	Parasite	Remarks
Dodecaceria concharum	Gut	*S. echinatum*, C. & M.*, 1899	Gametocyst and sporocysts known
Nerine sp.	,,	*S. pendula*, Giard, 1884	Type species; coelomic habitat first given is incorrect
Nerine cirratulus	,,	*S. pendula* in C. & M., 1899	C. & M. showed type-species is intestinal
Phyllodoce sp.	,,	*Selenidium* sp., Claparède, 1861	
Polycirrus aurantiacus	,,	*Selenidium* spp., Fantham, 1908	Original observation
Polydora coeca	,,	*Selenidium* sp., C. & M., 1899	
Polydora flava	,,	*Selenidium* sp., C. & M., 1899	
Pomatoceros triqueter	,,	*Selenidium* sp., C. & M., 1899	
Polymnia nebulosa	,,	*S. costatum*, Siedlecki, 1903	Also said to occur in coelom, rarely
Protula tubularia	,,	*Selenidium caulleryi*, Brasil, 1907	Schizogony described in detail
Pygospio seticornis	,,	*Selenidium* sp., C. & M., 1899	
Sabella spp.	,,	*S. sabellae*, Lank., 1863	
Salmacina dysteri	,,	*Selenidium* sp., C. & M., 1905	
Scolelepis fuliginosa	,,	*Selenidium* spp., C. & M., 1899, 1901	Two species, one with single myoneme in which schizogony is known; the other with numerous myonemes
Scoloplos muelleri	,,	*Selenidium* sp., C. & M., 1899	
Serpula contortuplicata	,,	*S. serpulae*, Lank., 1863	
Spio fuliginosus	,,	*S. spionis*, Kölliker, 1845	
Spio calcarea	,,	*S. spionis*, Léger, 1892	Notified by Léger as a *Platycystis*
Spio martinensis	,,	*S. spionis*, Kölliker, 1845	
Sipunculus nudus	,,	*S. sipunculi*, Dogiel, 1907	The original account of schizogony is probably incorrect
Petalostoma minutum	,,	*Selenidium* sp., Brasil, 1907	
Phascolosoma vulgare / *Phascolosoma elongatum*	,,	*Selenidiidae* 2 spp., Brasil & Fantham, 1907	Schizogony and encystment known

* C. & M. = Caullery and Mesnil.

4. Family **Merogregarinidae**, Porter.

Trophozoite non-septate, ovoid, with a small, definite epimerite shaped like the head of a lance. Myonemes restricted to the anterior, pre-nuclear region. Schizogony intracellular. Sporogony known in part. Spores octozoic.

One genus.

(1) Genus *Merogregarina*, Porter.

Schizont intracellular, uninucleate during growth, becoming multinucleate at the end of its growth. Number of merozoites produced

from a schizont is relatively small. Gametocytes free, with anterior longitudinal myonemes.

One species only known at present.

Parasite	Habitat	Host	Remarks
Merogregarina amaroucii, Porter	Gut	*Amaroucium* sp.	From Port Jackson, New South Wales

5. Family Aggregatidae, Labbé.

Schizonts are sub-epithelial (coelomic), and their nuclear multiplication does not begin till growth is complete. Merozoites arranged around masses of residual protoplasm. No association between gametocytes, but copulation between free anisogamous gametes. Schizogony in Decapod Crustacea, sporogony in Cephalopod Mollusca.

One genus, containing several species.

(1) Genus *Aggregata*, Frenzel (=*Eucoccidium*, Lühe).

With the characters enumerated above. Many species differentiated by Moroff (1908), often on physiological grounds. These species, as given by Moroff, are tabulated below.

Parasite	Habitat	Host	Location	Gametocytes	No. of Sporozoites in Sporocyst
Aggregata spinosa, Moroff	Spiral caecum	*Octopus* [sp ?]	Cavalière (Var.), Mediterranean	♀ 250—300 μ ♂ 120—170 μ	24
A. légeri, Moroff	,, ,,	*Octopus*	Cavalière	♀ 200—250 μ ♂ 120—170 μ	16
A. labbéi, Moroff	,, ,,	*Octopus*	Cavalière		Sporocyst ?
A. schneideri, Moroff	,, ,,	*Octopus*	Cavalière		Sporocyst ?
A. siedleckii, Moroff	,, ,,	*Octopus*	Cavalière		16
A. jacquemeti, Moroff	,, ,,	*Octopus*	Cavalière	♀ 100—150 μ ♂ 80—110 μ	16
A. octopiana, Schneider	Rectum	*Octopus vulgaris*		130—180 μ	16
A. duboscqi, Moroff	Spiral caecum	*Octopus*	Luc-sur-mer	80—100 μ	8
A. reticulosa, Moroff	,, ,,	*Octopus*	Cavalière	120—150 μ	Sporocyst ?
A. ovata, Moroff	,, ,,	*Octopus*	Cavalière	200—300 μ	Sporocyst ?
A. stellata, Moroff	,, ,,	*Octopus*	Cavalière		Sporocyst ?
A. eberthi, Labbé	,, ,,	*Sepia officinalis*	Cette; Trieste	90—120 μ	3 (Schizogony in *Portunus arcuatus* and *P. depurator*)
A. arcuata, Moroff	,, ,,	*Sepia*	Cavalière	120—140 μ	3
A. mingazzini, Moroff	Intestine	*Sepia*	Cette	120—150 μ	4
A. minima, Moroff	?	?	Mediterranean	50 μ	3 ?
A. frenzeli, Moroff	Spiral caecum	*Sepia*	Cette	80—100 μ	Sporocyst ?
A. mammillana, Moroff	?	?	Cavalière	100—150 μ	4

Of the following species, occurring in crabs, the schizogonic phases only are known.

Parasite	Habitat	Host
Aggregata portunidarum, Frenzel	Body cavity	*Portunus arcuatus*
		Carcinus maenas
A. coelomica, Léger	,, ,,	*Pinnotheres pisum*
A. vagans, Léger and Duboscq	,, ,,	*Eupagurus prideauxi*
A. inachi, G. Smith	,, ,,	*Inachus dorsettensis*
		Inachus scorpio
Aggregata sp., Léger and Duboscq	,, ,,	*Pachygrapsus marmoratus*

VIII. Classification.

(α) *Previous Classifications.*

The origin of the name *Schizogregarinae* (Léger, 1900) has already been set forth in an earlier portion of this paper (p. 370). Minchin, in his article on the Sporozoa (1903), adhered to the classification as set forth by Léger, but included *Gonospora longissima* in the Eugregarines, its original position. Caullery and Mesnil in 1898 had found merozoites in the gut of *Dodecaceria concharum*, a Polychaete which harboured the *Gonospora*, and Léger had suggested that *Gonospora* was possibly a Schizogregarine. Caullery and Mesnil associated the merozoites found by them with *Gonospora*, but in 1907 the question of the true adult, to which these merozoites belonged, was reopened by Brasil. The latter declared his belief that they were stages, not in the life-history of *Gonospora longissima*, but, rather, were part of the life-cycle of *Selenidium echinatum*, which also was a parasite of the gut of *Dodecaceria*. The question of the systematic position of *Gonospora* was incorporated by Brasil in an account of a new Selenidium, *S. caulleryi*, which he had discovered, and in his paper (1907) he advanced a new classification of the Schizogregarines. This was the first attempt to classify the Schizogregarines on a broad scale, and definitely introduced, in the *Selenidiidae*, forms with intraepithelial schizogony.

In the first family Brasil placed Schneider's genus *Ophryocystis*, and retained to some extent the historic name by styling the family the *Amoebosporidiidae*. *Schizocystis* he included with *Ophryocystis* in this family, in which he also placed *Eleutheroschizon*.

Brasil's second family was the *Selenidiidae*, marked by constancy of body-form and the presence of contractile myonemes on the body of the parasite.

The much discussed *Aggregata* (*Eucoccidium*) which sporulates in the gut wall of the cuttlefish and octopus, while its schizogony occurs in the crab, was placed by Brasil, on account of its widely different mode of life, in a separate (third) family, the *Aggregatidae.*

Quite recently, in July 1908, the paper of Léger and Duboscq on the *Aggregatidae* appeared, following on a long paper by Moroff. In this, Léger and Duboscq propounded a new classification of the Schizogregarines, based on the fact that in *Ophryocystis* two gametocytes give rise only to a single, octozoic spore. *Ophryocystis* is therefore placed in the subdivision *Monospora,* the remaining families being placed in the *Polyspora,* since, in the latter, two gametocytes associate, encyst and give rise to many gametes, each of which produces octozoic spores, as in the Eugregarines.

Léger and Duboscq's classification, including *Eleutheroschizon* and *Siedleckia,* appears thus in tabular form:

Schizogregarinae	*Monospora.*	*Ophryocystidae...Ophryocystis, Eleutheroschizon.*
	Polyspora	*Schizocystidae...Schizocystis, Siedleckia.*
		Selenidiidae...Selenidium.
		Aggregatidae...Aggregata.

In this classification the name *Amoebosporidia* is finally discarded, but unfortunately *Ophryocystis* and *Schizocystis,* which are alike in possessing extracellular schizogony, are widely separated.

The following important points in connection with Schizogregarines need to be considered in any scheme of classification:

(1) The extracellular character, as regards the tissues of the host, of *Ophryocystis* and *Schizocystis.*

(2) The intra-cellular character of the schizont in the *Selenidiidae* and the *Merogregarinidae.*

(3) The fact that the schizogony of the *Aggregata* occurs in a different host (Decapod Crustacean) from its sporogony, which takes place in a Cephalopod Mollusc. In the words of Léger and Duboscq, *Aggregata* is a Gregarine which is digenetic as regards phases of its life-cycle, and heteroïc as regards its hosts.

(β) *A New Classification.*

We must not overlook the uncertainty which still prevails regarding the phenomena of fertilisation in the so-called *Eucoccidium,* as described by Moroff (1908) on the one hand, and Siedlecki (1898) on the other, and its bearing on the position of *Aggregata* (*Eucoccidium*). Taking

all the points, noted in the foregoing paragraph, into consideration, it seems to me that *Aggregata* stands apart from the rest of the group in being heteroïc. This fact is not obvious in Léger and Duboscq's classification, and the mode of sporulation, on which they base their classification, can be easily explained (see p. 382). The method of sporulation does not seem to me to be of so much importance as the heteroïc character of *Aggregata,* especially when it is remembered that the presence of extracellular schizogony brings *Ophryocystis* and *Schizocystis* into contiguity.

Table showing the position of the Schizogregarinae in the Order Gregarinida.

Sub-order: **Schizogregarinae**—Gregarines with a schizogonic phase in their life-cycle.

Section I—**Homoïca**—Schizogregarines whose complete life-cycle takes place in a single host.

Sub-section (α) **Ectoschiza**—With schizont extracellular.
Ophryocystidae, with a single sporocyst.
e.g. *Ophryocystis.*
(?) *Eleutheroschizon* (sporogony unknown).
Schizocystidae, with numerous sporocysts.
e.g. *Schizocystis.*
(?) *Siedleckia* (sporogony unknown).

Sub-section (β) **Endoschiza**—With schizont intracellular.
Selenidiidae, with longitudinal myonemes the whole length of the body.
e.g. *Selenidium.*
Merogregarinidae, with longitudinal myonemes confined to the anterior (pre-nuclear) region.
e.g. *Merogregarina.*

Section II—**Heteroïca**—Schizogregarines whose life-history is divided between two hosts, with schizogony in the one, sporogony in the other.
Aggregatidae, in crabs and cephalopods.
e.g. *Aggregata.*

The Schizogregarines may therefore be conveniently divided into forms whose life-cycle is completed in one host, *i.e.* **homoïc** forms, in contradistinction to the **heteroïc** *Aggregata.* Among the homoïc forms

we have those with extracellular schizogony, *i.e.* **ectoschizous** forms, and those with intracellular schizogony (*e.g. Selenidiidae* and *Merogregarinidae*) which are **endoschizous.** I would, then, divide the *Schizogregarinae* into two new sections, viz. the *Homoïca* and *Heteroïca.* The *Homoïca* are divisible into two sub-sections, viz. the *Ectoschiza* and the *Endoschiza.* Including *Merogregarina* (Porter) this new classification is given in the accompanying table (p. 404).

IX. Affinities.

The position of the Schizogregarines in the general scheme of classification of the Sporozoa is clearly within the order *Gregarinida*, and the sub-class *Telosporidia* (Schaudinn). Further, the Schizogregarines belong to the interesting assemblage of animals known as annectant forms. They link the Gregarines with the Coccidia, for in their trophic and sporogonic phases the *Schizogregarinae* resemble the *Eugregarinae*, while in the presence of an asexual multiplicative stage in their life-cycle they resemble the Coccidia.

Before concluding, the interesting and unique form *Schaudinnella henleae* (Nusbaum) may be mentioned for the light it sheds on the possible evolution of the *Telosporidia. Schaudinnella* possesses distinctly gregariniform, trophic phases, separate gametocytes without association or encystment stages, and well differentiated gametes, resembling in form those of Coccidia. The zygotes form sporozoites directly without the intervening formation of sporocysts.

A gregariniform *trophozoite* has two courses open to it, either to become a *schizont* and by schizogony or multiple fission produce a crop of merozoites, or to become a *gametocyte* and pass through a process of gametogony, giving rise to gametes. In the Gregarines two gametocytes associate and form a common cyst. Then each gametocyte divides to form many gametes which conjugate in pairs, producing many zygotes or sporoblasts, the whole sexual process having taken place inside a gametocyst. On the other hand, in the Coccidia many gametes are formed from the male (micro-) gametocyte, but only one gamete from the female (macro-) gametocyte, and fertilisation occurs between free gametes which encyst *after* the zygosis. In *Schaudinnella* many microgametes are formed from each microgametocyte, but only a few (some 8 to 10) macrogametes from each macrogametocyte, and conjugation occurs between the free gametes. In the occurrence of conjugation between anisogamous, free gametes *Schaudinnella* more nearly resembles

a Coccidian, and is, indeed, intermediate in this feature between anisogamous Gregarines and the strict Coccidia. In most of the Schizogregarines the gametes are isogamous. *Schaudinnella* more nearly resembles the Coccidia in the particular characteristic (gamete formation) in which the majority of the Schizogregarines (leaving out *Aggregata* on which further information is required) differ from the Coccidia. *Schaudinnella* probably resembles a primitive type connecting the Gregarines and the Coccidia, devoid of schizogony (a differentiation evolved for the purpose of auto-infection of the host), yet already possessing the well differentiated gametes, characteristic of the Coccidia. From an ancestral, plastic form resembling *Schaudinnella*, the Eugregarines appear to have evolved on the one hand, and the Schizogregarines and Coccidia on the other; or perhaps, more correctly, the Schizogregarines (by acquiring schizogony) have evolved from the Eugregariniform type in the direction of the Coccidia. *Aggregata* would seem to link up the isogamous Schizogregarines and the anisogamous Coccidia.

Probably many other members of the Schizogregarines have yet to be discovered. It is possible that some of the Gregarines at present only known in the trophozoite phase may yet prove to have schizogonic stages, and their sporogony may occur at a strictly limited period of the year (see p. 390). There is here a wide field for research.

In conclusion it may be noted that the life-history of the Schizogregarines has a direct bearing on the advisability of retaining the separation of the Sporozoa into the *Telosporidia* and *Neosporidia* of Schaudinn, according as the reproductive phase of the life-cycle occurs at the end of or during the trophic or growing period.

The *Ophryocystidae* and *Schizocystidae* increase in volume during schizogony, and on this account would be placed in the *Neosporidia*, and not in the *Telosporidia* along with the *Eugregarinae*. Such a separation of the Schizogregarines and Eugregarines would be unfortunate, and scientifically unsound. Again, the *Microsporidia* are now placed among the *Neosporidia*, but they do not sporulate until they have completed their growth, which is a Telosporidian character. This is pointed out by Léger and Duboscq (1908, p. 101, footnote).

It seems preferable, then, to divide the Sporozoa, following Metchnikoff and Mesnil, into (a) *Ectospora*, wherein the spore-mother-cells or sporoblasts are formed at the periphery of the gametocyte, and (β) *Endospora*, in which the spore formation occurs in the interior of the body of the trophozoite, the spore-mother-cell or pansporoblast being separated off internally. The *Schizogregarinae* would then be

placed in the *Ectospora*, along with the *Eugregarinae*, the *Coccidiidea* and the *Haemosporidia*.

However, it does not seem profitable to discuss further the classification of the Sporozoa, on the basis of the life-cycle of the Schizogregarines. Classification is at the best only tentative, and must change with advancing knowledge. It is of much more importance to work out further *complete* life-cycles, and so—by filling in the gaps—to increase our knowledge of the facts which must underlie all classification.

X. SUMMARY.

1. The term *Schizogregarinae* Léger (1900) is the name now given to a sub-order of the *Gregarinida*, the remaining members of which are known as the *Eugregarinae*. The Schizogregarines were formerly known as *Amoebosporidia* Aimé Schneider (1884), a name given in misapprehension of the character of the cytoplasmic processes, fixative in function, present in the genus *Ophryocystis*. Two species of *Ophryocystis* (*O. buetschlii* and *O. francisci*) were the only members of this sub-order known before 1900.

2. At present the sub-order *Schizogregarinae* contains five families: *Ophryocystidae*, *Schizocystidae*, *Selenidiidae*, *Merogregarinidae*, and *Aggregatidae*.

3. All these organisms show well-marked schizogonic stages in their life-history, and—with the possible exception of the *Aggregatidae*—follow after the *Eugregarinae* in their methods of sporogony.

4. In *Ophryocystis* and *Schizocystis* the schizogony is extracellular, that is, these forms are **ectoschizous**. The life-cycle of the former is shown in Fig. 1. In these parasites the number of the nuclei in the schizont increases simultaneously with its volume.

5. In *Selenidium* and *Merogregarina* the schizogony is intracellular, in other words these forms are **endoschizous**. The life-cycle of the former is illustrated in Fig. 3. In these forms the schizont is uninucleate during its growth, only becoming multinucleate at the end of the growing period.

6. *Ophryocystis* forms only one sporocyst, a fact which has been emphasised by Léger and Duboscq (1908), by the placing of the *Ophryocystidae* in a special section, the *Monospora*. However, this apparent peculiarity is easily explained by a process of reduction and degeneration having taken place, affecting with one exception all the

gametes formed from each gametocyte. There is good morphological evidence in support of this explanation (see p. 382, and Fig. 4, D).

7. Figures of the interesting form *Schizocystis gregarinoides* (Léger, 1900) are not yet published, but a paper dealing with this organism is promised by Prof. Léger at an early date.

8. *Aggregata* differs from other Schizogregarines in that its schizogony takes place in one host (crab), while its sporogony occurs in another (Cephalopod mollusc). In this respect *Aggregata* resembles the *Haemosporidia*. The schizogonic phases in Crabs were formerly regarded as belonging to a gymnosporous Gregarine, *Aggregata* Frenzel, while the sporogonic phases were considered to belong to a Coccidian, *Eucoccidium* (*Benedenia*) in cuttlefishes and *Octopus*. Regarding this, Léger and Duboscq (1908, p. 102) write "...*Aggregata*, avec un changement de cycle coïncidant avec un changement d'hôte, c'est à dire qui soient à la fois digénétiques et hétéroïques."

9. It is evident therefore that the *Aggregatidae* stand apart. On this account, I suggest a division of the *Schizogregarinae* into two sections, termed respectively, the *Homoïca* (to include the first four families discussed in this paper, wherein schizogony and sporogony take place in the same host) and the *Heteroïca* (for the *Aggregatidae*).

10. Among the *Homoïca* we have extracellular schizogony (ectoschizous forms) in the *Ophryocystidae* and *Schizocystidae*, and intracellular schizogony (endoschizous forms) in the *Selenidiidae* and *Merogregarinidae*. This difference is not merely superficial, it requires to be emphasised, and for this reason I would divide the section *Homoïca* into two sub-sections, termed respectively *Ectoschiza* and *Endoschiza*.

11. The classification of the Schizogregarines, which I would propose, is as follows:

- **Schizogregarinae**
  - **Homoïca** (nov. sect.)
    - **Ectoschiza** (nov. sub-sect.)
      - *Ophryocystidae*
        - *Ophryocystis*
        - (?) *Eleutheroschizon*
      - *Schizocystidae*
        - *Schizocystis*
        - (?) *Siedleckia*
    - **Endoschiza** (nov. sub-sect.)
      - *Selenidiidae*..........*Selenidium*
      - *Merogregarinidae*...*Merogregarina*
  - **Heteroïca** (nov. sect.)........................*Aggregatidae*...... ...*Aggregata*

12. Much further research is needed on the life-cycles of the *Endoschiza*, especially among the *Selenidiidae*, which occur so frequently in the *Annelida*. Sporogonic stages are at present unknown in *Eleutheroschizon* and *Siedleckia*.

13. In connection with the *Aggregatidae,* and to a less extent with the *Selenidiidae,* stress is laid upon the necessity of carefully distinguishing between "coelomic" and "gut" parasites. (See pp. 397 and 387.)

14. The *Schizogregarinae* form a most interesting link between the *Eugregarinae* and the *Coccidiidea.*

References to Literature.

Fuller references will be found at the end of many of the papers quoted in the following list:

Brasil, L. (1907). Recherches sur le cycle évolutif des *Selenidiidae.* Grégarines parasites d'Annélides polychètes. I. La schizogonie et la croissance des gamétocytes chez *Selenidium caulleryi,* n. sp. *Arch. f. Protistenkunde,* VIII. 370—397, 1 pl.

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APPENDIX.

Glossary of Terms relating to Schizogregarines.

Anisogametes. Gametes showing sexual differentiation.
Example: macro- and microgametes of *Aggregata.*

Digenetic. Haviug two phases in the life-cycle, viz. *schizogony* and *sporogony.*

Ectoschizous. Signifying that the *schizont* occurs on the *outside* of the cells of the host, and is attached thereto by cytoplasmic processes. In such cases the schizont is **extracellular.**
Example: schizonts of *Ophryocystis.*

Endoschizous. Signifying that the schizont occurs *inside* the host-cell, that is, the *schizont* is **intracellular.**
Example: schizonts of *Selenidium.*

Endogenous reproduction. The formation by the parasite of *merozoites* destined to re-infect the host. (See *Schizogony.*)

Exogenous reproduction. The formation by the parasite of resistant spores destined to infect fresh hosts. (See *Sporogony.*)

Gametes. Conjugating individuals developed from *gametocytes*, and giviug rise to *zygotes.*

Gametocyte. The adult *trophozoite* matured for the production of *gametes.*

Gametogony. The process of gamete-formation.

Gymnospore. "Naked spores": not enclosed in a protective covering.

Heteroïc. When two differeut hosts are required for the evolution of the complete life-cycle of a parasite.
Example: *Aggregata.*

Homoïc. When the life-cycle of a parasite is completed within one host.
Examples: *Ophryocystis*, *Selenidium.*

Isogametes. Gametes which are morphologically similar.
Example: *Ophryocystis.*

Karyosome. A nuclear corpuscle, containing a certain amount of chromatin in its substance, thereby differing from a nucleolus.

Merozoite. A free-moving uninucleate individual resulting from *schizogony.* A merozoite is often club-shaped.

Plasmotomy. The breaking-up of a multinucleate Protozoön into a number of portions or daughter-forms, each containing a variable number of nuclei.
Example: *Siedleckia.*

Schizont. A full-grown *trophozoite* which has exhausted its host-cell and is about to multiply asexually, that is, divide directly into numerous uninucleate parts (*merozoites*).

Schizogony. A simple form of *sporulation* in which a *trophozoite*, without encysting, breaks up into numerous uninucleate masses of protoplasm termed *merozoites.* This process is sometimes called *asexual multiplication* or *endogenous reproduction*, and serves to increase the number of parasites in the host (auto-infection).

Sporoblast. A uninucleate mass of protoplasm arising from a *zygote.* A sporoblast gives rise to *sporozoites.*

Sporocyst. The tough chitinoid membrane secreted on the outer surface of a *sporoblast.* A sporocyst enclosing eight sporozoites is often termed an *octozoic spore.*

Sporogony. The formation of resistant spores from a *zygote*, following upon a sexual act. This process is sometimes termed *exogenous reproduction*, and serves for the infection of new hosts (cross-infection).

Sporozoite. A fine, curved, falciform, naked mass of protoplasm formed from a *sporoblast.* The sporozoite is the agent which starts an infection.

Sporulation. A method of rapid multiplication by the formation of reproductive bodies (*spores*), each of which is a fragment of the parent body.

Trophozoite. An individual Sporozoön during its *trophic* phase, that is, during the time the parasite is absorbing nutriment from its host and is growing rapidly. The trophic period is one of " vegetative " growth.

A trophozoite may become either a *schizont* or a *gametocyte.*

Zygote. The individual resulting from the fusion of two *gametes.*

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AND
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